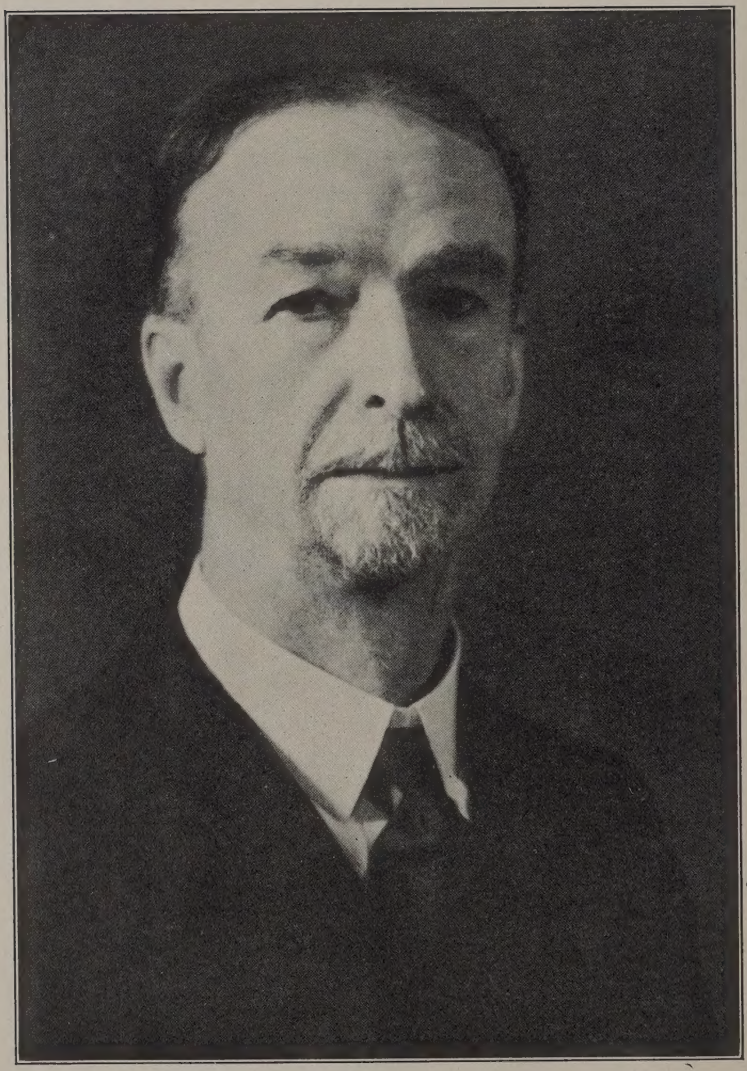

THEOBALD SMITH (1859–1934)

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THEOBALD SMITH AS A PARASITOLOGIST

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The name of Theobald Smith is associated with some of the most striking and outstanding discoveries in the field of parasitology in the past century, discoveries of great medical and economic importance. Of these the two most outstanding were in connection with Southern cattle fever and with blackhead in turkeys. Other investigations by Smith include work on a blood parasite of turkeys, on cattle, sparrow, rabbit and mouse coccidia, an intestinal sporozoan in cattle, mouse sarcosporidia, a rabbit sporozoan, an amoeba in swine, and worms in cattle, observations on mosquitoes and house flies, and general observations on parasitism and on immunity in parasitisms.

Theobald Smith was probably best known for his work on Southern cattle fever, work of outstanding importance for its medical and economic significance, and for its value in paving the way for many subsequent scientific investigations along the same lines. Unfortunately, the historical record of the work on Southern cattle fever has been badly kept in most references to that work, despite the fact that the early published reports contained a fairly exact record. The widely circulated version published by de Kruif presents the case very badly, and is very unfair to many of Dr. Smith's associates in the Bureau of Animal Industry. The present writer takes this occasion, in discussing Smith as a parasitologist, to publish a more precise record of Smith's work, and to correct some of the errors which have been widely circulated in print. One of the most common misstatements is to the effect that Smith first demonstrated the transmission of other parasites by arthropods, and another is that he first demonstrated the transmission of Southern cattle fever by ticks.

The historical background of the work on Southern cattle fever is as follows:

1821. Cattle fever tick described under name of *Ixodes annulatus* by Say.

1867. Development of mouse spirurid, *Protospirura muris,* in mealworm, *Tenebrio molitor,* reported by Leuckart.

1869. The parasite of Southern cattle fever first noticed by R. C. Stiles (Tr. N. Y. St. Agric. Soc. 1869 (1867), v. 27, pt. 2, pp. 995, 1134–1145); see Smith (1889; Med. News, Phila., v. 55 (25), pp. 689–692) for comment.

1869. Development of double-pored tapeworm, *Dipylidium caninum,* in biting dog louse, *Trichodectes latus,* reported by Melnikow.

1871. Development of Guinea-worm, *Dracunculus medinensis,* in *Cyclops* reported by Fedtschenko.

1878. Development of Bancroft's human filarid, *Wuchereria bancrofti,* in mosquitoes reported by Manson.

1885. Northern line of cattle tick area established by D. E. Salmon, Chief, Bureau of Animal Industry.

1888. Babes (Compt. rend. Acad. d. sc., Paris, v. 107 (18), Oct., pp. 692–694) reported that an enzootic hemoglobinuria of cattle in Roumania was due to a bacterium about 0.5 μ in diameter, divided into 2 parts by a striation in the middle, and sometimes divided into 4 parts by a transverse striation, the parasites occurring on or in the red blood cells.

1889. Babes (Arch. f. path. Anat., Berlin, 11 s, v. 5 (1), Jan., pp. 81–108) extended his communication of the previous year, referring to his organisms as "Haematokokken," and giving colored illustration of what are unmistakably piroplasms.

1889. Curtice (4th & 5th Ann. Repts., Bu. Anim. Ind., p. 436) notes: "The oft-repeated experiment of allowing native cattle to live on the trail of Texas cattle. . . . initiated on the 3d of July in the Chicago stockyards, of placing five cows in pens frequented by Texas cattle, with the resulting illness of all and the death of four from the Southern cattle fever."

1889. Kilborne (See Smith, 6th & 7th Ann. Repts. Bu. Anim. Ind., p. 95) exposed one lot of Northern cattle (probably from Maryland; experiments carried out at Bureau of Animal Industry Experiment Station at Bennings, D. C.) to North Carolina cattle after removing all ticks from the latter, and a second lot of Northern cattle to North Carolina cattle on which ticks were left. The native cattle in the first lot showed no disease; those in the second lot died of Southern cattle fever. This experiment was planned by Dr. Salmon and was carried out by Dr. Kilborne under orders from Dr. Salmon. Dr. Salmon stated this to one of his associates, who is my authority for the statement.

1889. Salmon (1st. Rept. Sec. Agriculture, pp. 90–91) reports the finding, in the Bureau of Animal Industry [by Smith], of peculiar bodies in the red corpuscles of cattle having Southern cattle fever, which bodies are micro-organisms but not bacteria and are associated with the destruction of blood corpuscles, the disease being essentially a blood disease.

1889. Smith (Med. News, Phila., v. 55 (25), pp. 689–693) reports

the bodies in the red cells, notes that R. C. Stiles had probably had the same thing in 1867, and compares his observations with those reported by Babes earlier in 1889. He thinks his organism is different from that of Babes, because Babes said he cultured his and also found it outside the red cells; despite these incidental errors of Babes, his figures show that he and Smith were both dealing with piroplasms.

1891. Smith (1891, 6th & 7th Ann. Repts. Bu. Anim. Ind., pp. 93–98) reports the joint experiments with Kilborne in 1890 to test more accurately the theory suggested "At various times and in different parts of the country. . . . that the ticks were the cause of Texas fever in northern cattle." Separate fields were arranged as follows: One contained ticky North Carolina cattle; one contained Texas cattle; one contained North Carolina cattle without ticks; one contained cattle ticks; one contained soil from infected North Carolina farm pastures. Among native cattle exposed on these fields, there were no cases of fever in the pasture containing North Carolina soil, but ticks were washed from the field containing fever ticks into the field containing Carolina cattle without ticks, and native cattle in this field, as well as in the fields with ticks or ticky cattle, all developed cattle-tick fever. Subsequently ticks were shipped from North Carolina, and the young ticks which were the progeny of these ticks, were put on 4 head of cattle; all 4 head became sick of cattle fever, and 2 died, 1 was killed, and 1 recovered. In the case of the first of these 4 animals, it was still not thought that ticks were directly responsible for the disease, and Smith and Kilborne (1893 Bu. Anim. Ind. Bull. 1, p. 105) state that ticks were put on the animal to ascertain their effect on the number of blood corpuscles.

1891. At this same time (1891) Smith reports that the organism of cattle tick fever is a protozoan.

1891. Curtice presented a paper outlining the life history of the cattle fever tick before the Washington Biological Society on Feb. 3, 1890 (Curtice, 1891, J. Comp. Med. & Vet. Arch., v. 12 (7), July, pp. 313–319). In the published paper cited here, Curtice notes: "Having learned to breed the ticks in quantity, I conceived the experiments of taking Northern calves and placing young ticks upon them to determine the extent and kind of injury that the ticks cause their hosts. These experiments were planned for the summer of 1890, and were sanctioned by Dr. D. E. Salmon, the Chief of the Bureau of Animal Industry, where I was then employed. I, however, was sent to another field [This was a paleontological field trip for the Smithsonian Institution. M. C. H.] but not to the detriment of the experiment, for I found on my return that they had been carried out on quite a large scale as detailed in the report of the Secretary of Agriculture for 1890 (p. 109)." Curtice

(conversations with Dr. Mohler and with the writer) outlined the proposed experiments to Dr. Kilborne as well as to Dr. Salmon.

1891. In this same paper, Curtice proposed the genus *Boophilus* for the cattle fever tick.

1892. Curtice (J. Comp. Med. & Vet. Arch., v. 13 (1), pp. 1–7) suggests that animals should be freed from ticks so far as possible, and expresses the belief that this will "so free the pasture that what at first would seem a formidable task would grow easier and easier." In 1896 he urged tick eradication over the State of Virginia and over the United States. In 1899 (See Bu. Anim. Ind. Circ. 187, p. 255) he inaugurated a tick campaign in North Carolina, and his belief, in which he stood almost alone, that ticks could be eradicated, together with the inauguration of the campaign against them by Curtice led to his being termed "The father of tick eradication." His work in North Carolina was continued by Tait Butler. Mohler, now chief of Bureau, worked on tick eradication for the Bureau in Virginia in 1904, and the Bureau started its nation-wide campaign in 1906.

1893. Piroplasms first named as *Babesia bovis* by Starcovici, and as *Pyrosoma bigeminum* by Smith and Kilborne.

1906. N. S. Mayo (Breeder's Gaz., v. 49 (11), p. 564) recommended very highly the arsenical dip, which had been used to some extent in South Africa, South America and Australia against ticks, as very effective in destroying cattle ticks.

1912. Ransom and Graybill (Bu. Anim. Ind. Bull. 144, 65 pp.) standardized the arsenical dip so that it would kill ticks but would not injure cattle, the chemical work being done in the Biochemic Division of the Bureau of Animal Industry.

1914. Chapin (U. S. Dept. Agric. Bull. 76: 17 pp.) reports a field test outfit for testing arsenical dips at the vats to maintain standard strength in the course of dipping.

From this background of matters relating to the transmission of Southern cattle fever by the cattle tick, one may summarize events as follows:

As regards the tick, it was first described and named by Say and allocated to its present generic status by Curtice, and its life history was worked out by Curtice. Curtice's work on the life history made possible the critical experiments on tick transmission of Southern cattle fever.

As regards the organism of piroplasmosis, it appears to have been seen first by R. C. Stiles, described first as associated with a cattle fever by Babes, and was described and named independently by Smith and Kilborne and by Starcovici in the same year.

As regards transmission of animal parasites by arthropods, this had

been reported for nematodes and cestodes by Leuckart, Melnikow, Fedtschenko and Manson, from 1867 to 1878.

As regards transmission of piroplasmosis, there was a wide-spread belief among stockmen that cattle ticks could carry Southern cattle fever, various experiments had shown that Northern cattle acquired the disease from contact with Southern cattle and with premises used by them, Kilborne carried out the first significant experiments in 1889, Curtice had planned the experiments involving placing seed ticks, the progeny of adults taken from Southern cattle, on Northern cattle, and the Chief of the Bureau of Animal Industry had approved his carrying out these experiments in the summer of 1890, and Smith and Kilborne actually carried out these critical experiments that summer.

As regards control, Salmon had established the northern line of the tick area in 1885, Curtice suggested that ticks be eradicated as early as 1892, and started eradication soon after in North Carolina, various workers in different parts of the world developed the arsenical dip, Ransom and Graybill standardized the dip, and Chapin devised the field test outfit essential for a tick-eradication campaign.

Quite precisely, then, Smith was the man who connected the occurrence of a piroplasm, as the cause of bovine hemoglobinuria, with the transmission of the piroplasm and the disease by the cattle tick. In this work his observations on the piroplasm were independent, although they followed those of Babes in time of publication, and it appears from at least two of his papers that he had seen and studied Babes' paper; his critical experiments on transmission were planned by Curtice and approved in advance by Salmon, and were carried out in cooperation with Kilborne. Smith's share in this work was actually what the world at large believes it to be, a large and important one. So far as the present writer can ascertain, Smith, in his published papers, never claimed for himself more than is credited to him here. Smith, like scientists in general, built on the work of others, and received direct aid from others, and his work was continued and extended by others. His credit is not impaired by giving full credit to the other scientists who preceded him, worked with him, and followed him.

Now let us see what the most highly publicized version of Smith's work, that of de Kruif (1926; Country Gentleman, January, pp. 18, 19, 59), claims for Smith and says about his associates.

"Counting Smith, there were four members of the staff of this bureau [Animal Industry]. The chief was a good man named Salmon. He was enthusiastically interested in what germs might do to cows. . . . But he knew nothing of how to find the microbes harassing these valuable creatures." Actually Dr. Daniel Elmer Salmon was a scholar, a scientist, and an unusually able administrator. He was educated in veterinary

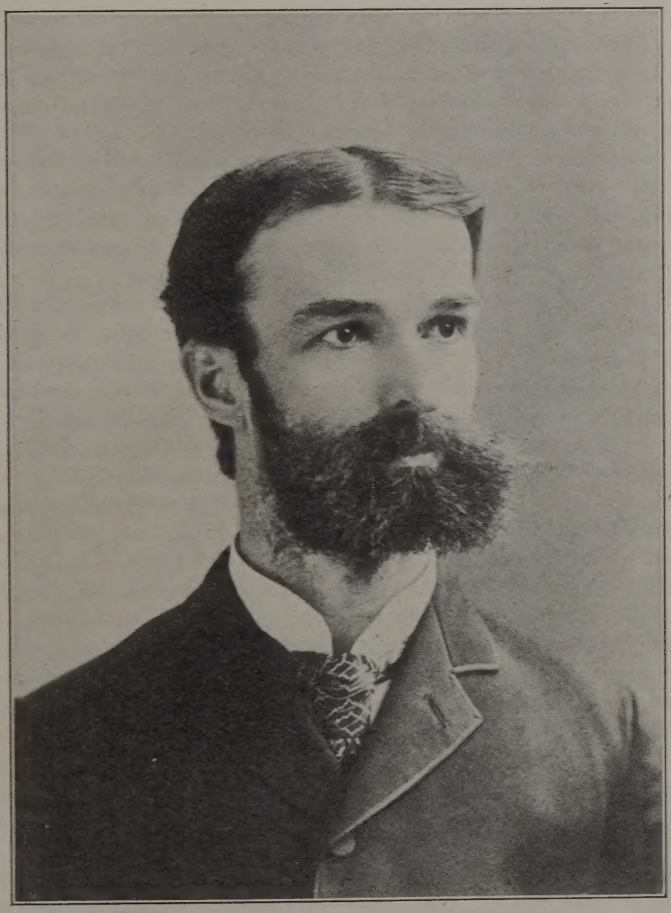
medicine at Cornell and at Alfort, France, receiving the degree of D. V. M. from Cornell in 1876. He entered the Department of Agriculture in 1881, and established the Veterinary Division in 1883 and the Bureau in 1884. Smith received the degree of M. D. at Albany Medical College in 1883, and was brought to the new Bureau in 1884, by which time Salmon had published a dozen papers in the field of parasitology, aside from papers in other medical fields. Salmon directed the research of his Bureau in no uncertain fashion, and the men who knew him, Mohler, Curtice, Stiles, Ransom, Hassall, Schroeder, Cotton, Moore and others, have always expressed to me a high regard for his intellectual ability. His selection of his staff, such as Smith and the men named above, shows that he had one of the requisites for a good administrator, the ability to select competent associates. His record as Chief of Bureau includes, among other things: The organization of his Bureau along sound lines of research and regulatory activities; the eradication of contagious pleuropneumonia of cattle; the study and control of Southern cattle fever; the establishment of the Federal meat inspection service; the establishment of inspection of exported animals and the ships carrying them, thus doing away with the cruelties which had characterized this trade and greatly reducing the losses of animals; and the establishment of a quarantine to keep from this country diseases which would otherwise be imported. This is the man who, according to de Kruif, knew nothing of how to find microbes.

"Then there was Mr. Kilborne, who rejoiced in the degree of Bachelor of Agriculture and was something of a horse doctor." Dr. Kilborne was Superintendent of the Bureau's Experiment Station from 1885 to 1894. The present writer, who might also be called "something of a horse doctor," invites attention to the record of Kilborne's part in first showing the transmission of Southern cattle fever by ticks in 1889, which work was done, according to Dr. Cooper Curtice and Dr. C. W. Stiles, by order of Dr. Salmon and not by order of Dr. Smith, and which was the basis for Dr. Smith's subsequent connection with the critical transmission experiments, as a reason why this slurring reference by de Kruif to Kilborne is not in order.

"Theobald Smith had what so many of those mystified scientists and baffled horse doctors lacked, horse sense." The historical record already given is adequate answer as to whether Smith's associates had "horse sense" or were merely mystified and baffled. They cleared the path for Smith and they and others continued his work. Without them and others, Smith's work would have been impossible. Their reward, as bestowed by de Kruif, seems somewhat short of adequate.

"One day Kilborne told him (Smith) of the cattlemen's ridiculous theory about the ticks. In a moment he pricked up his mental ears."

The tick theory was very old. A committee on Southern cattle fever, headed by Gamgee of England, had reported in 1868, over 20 years previously: "That there is not the slightest foundation for the view that the ticks disseminate this disease." In effect, de Kruif is charging Dr. Smith with having worked for over four years on Southern cattle fever without ever having familiarized himself with the important literature on the subject or with the common beliefs in regard to it. Smith (1891) refers to the tick theory as suggested at various times and in various parts of the country. It is simply incredible that Smith could be so



THEOBALD SMITH IN 1889

thoroughly insulated from the subject he was investigating as de Kruif implies, and one must assume, in defense of Dr. Smith, that de Kruif did not get this statement from Dr. Smith or must have misunderstood him. In talking this matter over with Stiles, Curtice, V. A. Moore and others, I have been told by one of them that Dr. Salmon stated to him that he told Dr. Smith to test the theory of tick transmission before Kilborne actually tested it, and that Dr. Smith protested that it did not deserve test. Whether this is true or not, de Kruif's statement reflects on Smith, in spite of the fact that de Kruif evidently did not intend a reflection. Smith himself (1891: 6th & 7th Ann. Repts. Bu. Anim. Ind., p. 95) says: "Dr. F. L. Kilborne. . . . conceived the happy idea of testing this popular theory of the relation of ticks to the disease."

"Smith, with a government bug expert named Cooper Curtice, kept himself busy with vast studies of the lives and works and ways of ticks." This "government bug expert" had the degrees of B.S., M.D. and D.V.S. He was and is well known for his excellent scientific research in parasitology, and was the pioneer in tick eradication and bovine tuberculosis eradication in this country. He worked out the life history of the cattle tick, using tick eggs which Smith had found in a bottle into which live females had been dropped.

In the rest of de Kruif's story there is a confusion of the rôles played by various workers and of the sequence of events. It is very entertaining reading, but in supplying Smith with words and ideas de Kruif has substituted inaccuracies for accuracy. In casting aspersions on everyone except Smith, de Kruif has made the same mistake that he made when he entered the Grassi-Ross controversy instead of telling just what Grassi did. The controversies among scientists usually contribute nothing to their cases; the scientific record stands as published and those who wish to ascertain the facts may do so. Usually the fact is that a man deserves credit for what he did; all attempts to say that this or that is more important than something else, or that one man deserves more credit than another, are misguided attempts. So far as Smith is concerned, he contributed to our knowledge of piroplasms and their transmission by ticks, matters of great medical and economic importance, and he was ably assisted by his associates. Each worker was entitled to credit for his share of the work.

An authoritative statement of the facts at issue is contained in the Proceedings of the American Veterinary Medical Association, covering the Atlantic City meeting in 1901, these proceedings being published in 1902. From that report I quote the following:

Dr. Salmon. "The credit for discovering that Texas fever was transmitted by ticks has been given most of all to Dr. Theobald Smith, and, secondly, to Dr. F. L. Kilborne. I know all the facts, and want to say

that Dr. Smith does not deserve any credit for that part of the investigation. . . . The fact that he discovered the pathogenic organism in the blood had a tendency to convince him that the ticks had nothing to do with the production of the disease, and he was unwilling to spend his time in experiments to prove whether the ticks had anything to do with it or not. I was convinced from the coincidence of the quarantine district and the home of the tick that there was some connection between them. . . . I . . . called Dr. Kilborne . . . into my office, and instructed him to plan experiments. Acting on that positive instruction, Dr. Kilborne planned the experiments . . . and to Dr. Kilborne should be given the credit of carrying out these investigations."

Dr. Curtice. "My name has been introduced here as one who has had something to do with experiments in connection with the tick. . . . I do believe that Dr. Salmon had it in mind that perhaps ticks had something to do with it. . . . But he knows as well as I do that we had talks upon that subject before his instructions were given to Dr. Kilborne. . . . I had talks with Dr. Kilborne over and over again, arguing the tick theory, and telling him how easy it was to prove it. I know that I asked Dr. Salmon to have four animals set aside in four pens. I was sent to California . . . and the experiment was placed in Dr. Kilborne's hands. . . . We were together and talked over these things. Dr. Salmon is right in saying that Dr. Smith at first combated the theory. I well remember the night in the Biological Society when I stood up and asserted that the ticks had something to do with it, and Dr. Smith argued against it. . . . I worked on the life history of the tick. . . . Dr. Smith came to me one day and said: 'If you had counted the blood corpuscles you would have been the first to show that the ticks produced disease in the calf you experimented on.' "

It is interesting to note that although his rôle was primarily that of a pathologist and a pioneer in the young science of bacteriology, Dr. Smith did his two most striking pieces of work in the older field of parasitology. His first years of work on Southern cattle fever were devoted to a bacteriological and pathological study of this disease, ending in finding it to be due to a protozoan. When he subsequently encountered blackhead in turkeys, he was prepared by his experience with Southern cattle fever to recognize this new disease as a protozoan disease, and his first paper on blackhead in 1895 (Bu. Anim. Ind. Bull. 8, pp. 7–38) described the disease and gave its cause as a protozoan which he named *Amoeba meleagridis* and which Tyzzer later called *Histomonas meleagridis*. This paper gives a very good description of the clinical conditions and lesions and of the organism, and most of the paper is still orthodox and accepted doctrine.

This work alone would have ensured Smith a permanent and high

place in connection with blackhead, but he was destined to add something more to this subject. In 1917 (J. Exp. Med., v. 25 (3), pp. 405–414) he returned to the subject with a study of control measures. In 1920, Graybill and Smith (J. Exp. Med., v. 31 (5), pp. 647–655) published a paper of great scientific and economic interest. In a series of experiments with turkeys, they found that feeding feces from infected turkeys would not produce the disease in susceptible turkeys, that feeding feces from infected birds and simultaneously feeding embryonated eggs of *Heterakis papillosa* (=*H. gallinae*) would produce the disease, and, more surprisingly, feeding embryonated eggs of *H. papillosa* recovered from blackhead cases would produce the disease. Later in the same year, Smith and Graybill (1920;; J. Exp. Med., v. 32 (2), pp. 143–152) reported the transmission of blackhead to chickens by feeding embryonated eggs of cecum worms from chickens kept in an enclosure with turkeys. These findings, subsequently confirmed and extended by Tyzzer and others, constitute the first record of the transmission of a protozoan disease by a worm parasite, and still stand as unique. The only comparable observation is that of Simms, Shaw and Donham at Corvallis, Oregon, to the effect that salmon poisoning of dogs, which at present appears to be a virus disease, is transmitted by the salmon-poisoning fluke, *Troglotrema salmincola*. Obviously, this subject of the transmission of protozoan parasites by helminth parasites has not yet had adequate consideration, and it is still probable that further investigations will show that the blackhead parasite is not the only protozoan that has taken advantage of the possibility of utilizing helminths to supply transport from an infected host to a susceptible host. Here, as in the work on piroplasmosis, Smith had able assistance, and Graybill's contribution to this work was recognized by Smith in placing him on the first paper as senior author.

It is hardly to be expected that all of Smith's research in parasitology would have had the far-reaching consequences of his work on Southern cattle fever and on blackhead. Like much scientific work, some of his observations were of the incidental sort that takes advantage of an opportunity to make limited studies that cannot be followed up for lack of time or opportunity. These observations are covered here very briefly.

In his first paper on blackhead, Smith (1895) described an incidental finding, the presence of a protozoan parasite in the blood of some turkeys. This parasite was subsequently described by Laveran and Lucet as *Leucocytozoon smithi,* being dedicated to its discoverer. It was found to be widespread in Europe, but appears to have had no further consideration in the United States until 1929, when it was reported by Volkmar. In 1932, Skidmore showed that it was transmitted by *Simulium occidentale.* Here again we find Smith as the discoverer of a highly pathogenic proto-

zoan transmitted, as in the case of the piroplasm, by an arthropod parasite.

In connection with a further study of blackhead, Hadley's theory that *Amoeba meleagridis* was a stage in the life history of a coccidium was considered by Smith and Smillie (1917; J. Exp. Med., v. 25 (3), pp. 415–420). They examined 54 sparrows taken around a turkey yard and found 80 per cent infected. No connection with the blackhead organism was found.

Smith and Graybill (1918; J. Exp. Med., v. 28 (1), pp. 89–108) gave a clinical and pathological report of coccidiosis of cattle, together with a discussion of the coccidia, and stated that no reports of the disease in the United States had previously been published. However, in the same paper they note that Schultz had reported it from the North-West in 1915, and that slides submitted by him showed coccidia. He published a paper on the subject this same year (1918). In fact, Schultz was insistent in regard to the importance of coccidia in cattle, and the present writer had the impression from Schultz's papers at the time that Schultz had rather overemphasized the subject; perhaps he had not.

In 1889, Smith reported the occurrence of coccidia in the renal epithelium of the mouse. In 1902, Smith and Johnson (J. Exp. Med., v. 6 (3), pp. 303–316) described this as a new genus and species, *Klossiella muris*.

Smith and Florence (1925, J. Exp. Med., v. 41 (1), pp. 25–36) published a paper on *Encephalitozoon cuniculi* as a kidney parasite of the rabbit, discussing the parasite and its life history and the pathology of the disease associated with it.

In 1893, Smith (Bu. Anim. Ind. Bull. 3, pp. 73–78) published a note on a sporozoan found by him in the intestinal villi of cattle. In 1919, Railliet named this species *Gastrocystis smithi,* dedicating it to its discoverer.

In 1901, Smith reported his observations on the transmission of *Sarcocystis muris* by feeding infected muscle tissue from mice to other mice. In 1905 (J. Exper. Med., v. 13 (4), pp. 429–430), he reported the subsequent transmission of the protozoan from mouse to mouse for a period of 7 years. Here again Smith was a pioneer in working out the life history of Sarcocystis, his work having been confirmed and extended by subsequent workers.

In 1910, Smith (J. Med. Res., v. 23 (3), pp. 407–415) discussed the protective reaction of the host to intestinal coccidiosis of the rabbit. He found coccidia beneath the epithelial layer, each coccidium being surrounded by a close-fitting capsule, and interpreted this as evidence of a form of phagocytosis. During the past ten years the defensive reactions of hosts against parasites, especially along the lines of immunology,

have received much attention, but at the time Smith was interpreting the pathologic findings in coccidiosis in terms of defense reactions very little was being done on immunity against animal parasites. The subject is still in a state of flux, with the basic principles still to be formulated for the most part.

In 1910, Smith (J. Med. Res., v. 23 (3), pp. 423–432) reported a study of amebiasis in natural cases in swine. Amoebae were found in the ulcerative and necrotic areas of the mucosa but not in the intact mucosa. No experiments are recorded, and the work is primarily a study in pathology.

On at least two occasions, Smith discussed the finding of helminth parasites in association with lesions observed by him. In 1894 (Bu. Anim. Bull. 7, pp. 124–126), he reported larval nematodes from nodules in the small intestine of cattle. Presumably these worms were the common nodular worm of cattle. In the same paper, he reported the finding of pentastome larvae, *Linguatula rhinaria,* in the mesenteric glands of cattle. In 1910 (J. Med. Res., v. 23 (3), pp. 417–422), he reported the cattle filarid, *Setaria labiato-papillosa,* trapped and encysted in the mesentery of a tuberculous cow, with a second case from a tuberculous calf. A specimen of the same sort is in the parasite collection of the Bureau of Animal Industry. In the first animal he found a nematode in a nodule in the lung, but the specimen did not permit of an identification. Other nematodes, not identified, were found in the Peyer's patches of a calf. On these limited data Smith has speculated briefly as to the possible mechanism leading to the destruction of the parasites. What we know at present does not permit of much more extensive speculation.

In the field of entomology, Smith published observations on *Anopheles* in the vicinity of Boston, and on house flies as carriers of infectious diseases.

The above does not include all of the observations made by Theobald Smith in the field of parasitology, but the little that is omitted would add nothing to the discussion of his position as a parasitologist. Enough has been given here to establish the fact that the common conception of his importance in this field is sound. It is unnecessary to detract from the work of others in order to make Smith an outstanding figure; he was that of his own right and by virtue of his own achievements.

Theobald Smith's general observations on the subject of parasitism are not discussed in this paper. He incorporated them in a fairly large volume published not long before his death, and the writer will discuss them in a review of that book, since they are not the sort of thing that can be covered briefly within the scope of this paper. Theobald Smith was a pathologist and bacteriologist, primarily, and in spite of his numerous and important contributions to parasitology, was not a parasitologist

in the sense that the writer or anyone else who has devoted his adult career to parasitology is a parasitologist. The distinction is perhaps illustrated by a remark that Dr. Smith made to the writer in conversation on one occasion, to the effect that it was regrettable that the work in parasitology in the Bureau of Animal Industry was not put in the Pathological Division of that Bureau at the start and kept there. To his mind, parasitology was just a subdivision of pathology. It is a legitimate point of view, but no more legitimate than the view that parasitology is a phase of zoology. Parasitology is actually a phase of medical zoology. Nature has drawn no line between our sciences in the natural set-up, and we draw our lines merely from some selected point of view. Smith's point of view in this conversation, as in his book, is that of the medical man, rather than that of the parasitologist who sees both the zoological and the medical aspects, and who rates his subject, because of its wide scope and the specialized point of view established by an interest in parasitism, as worthy of an independent status by itself instead of a status subordinated to pathology, medicine or zoology. Smith was not an orthodox parasitologist and his brilliant success in the field of parasitology is somewhat comparable to that of the unorthodox Pasteur, a chemist who was brilliantly successful in the field of medicine.

STUDIES ON NORTH AMERICAN CERCARIAE[1]

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From the time of Joseph Leidy to comparatively recent years American larval trematodes and trematode life histories have received increasing attention. Before Cort published his pioneer work on larval trematodes of Illinois, very little was known about these larvae in North America. Up to 1914 only twelve cercariae had been reported from North America, but in the same year Cort added fourteen new species to this list. Now the number of described cercariae in this country has reached such proportions that it is not practical even to refer to all previous descriptions in this paper. Descriptions from the state of Illinois alone, including those in the present paper, cover thirty-eight species.

Regarding attempts to establish life histories of these forms, either experimentally or morphologically, many errors have been made, largely because of unsubstantiated generalizations or insufficient experimentation. I believe Dollfus (1914) was right when he stated that cercariae very similar in structure dwell in different hosts and have very different kinds of development, and that cercariae very different morphologically live in identical environments, and have similar courses of development.

Lebour (1912) used the development in sporocysts or rediae of these forms as a basic factor in her scheme of classification. Viewing this plan now in the light of numerous additions to taxonomic lists of cercariae from various parts of the world, I realize that such a scheme can denote only very general relationships since individuals of the subgroups within each of these two large groups differ greatly in most other respects.

No one can doubt that an accurate classification of cercariae based purely on relationship would be of great value in indicating possible clues to the life histories of closely related larvae. However, the still inadequate knowledge concerning the value of many larval characters in revealing adult relationships and the inadequate descriptions of many cercariae make such a classification still impossible for all of the described larvae.

Certain larval structures, such as the number and position of the penetration glands, the shape of the bladder, the branching of the ex-

[1] Contributions from the Zoological Laboratory, the University of Illinois, under the direction of Henry B. Ward, No. 464. This work was done under the direction of Professor H. B. Ward, to whom the writer wishes to acknowledge indebtedness for the use of his valuable private library and for his suggestions and helpful criticisms.

cretory system, the shape and and size of the stylet, and the character of the germinal mass, should be regarded as less important for definite specific diagnoses and more important for showing subgroup relationships.

Perhaps the statement of Faust (1917) shows adequately the viewpoint taken by some more recent workers, when he says: "The writer has attacked this part of the problem with the idea in mind that not only the fundamentals of the adult trematode are found in the mature cercaria, but that even the main descriptive features of the adult trematode are already present, so that the worker can recognize the adult in the larva." After various studies on many cercariae I am inclined to depart somewhat from the above viewpoint, for while many of the larval characters resemble closely those of the adult, yet I find several incompletely developed structures in the cercaria which bear little resemblance to the same structures in the adult. The character of the excretory system may change, due to the division of the flame cells and the growth of tubules and capillaries, and even a ventral sucker may appear during the metacercarial stage (McCoy, 1929). I believe the above must be considered carefully if we are to progress toward the use of dependable characters of the cercariae for specific diagnosis.

Similarity between excretory systems has proven to be of great value in determining relationships but I find this similarity too great to have the systematic significance given it by some workers. Several instances of different species of cercariae, as well as different species of adults are on record in which the excretory systems are identical as to the arrangements of the tubules and terminal cells.

As pointed out in my earlier paper (1930), technique methods do not insure a similarity in the proportions of body structures before and after killing and fixing. Obviously the study of preserved material cannot give a true and complete concept of these forms.

For these reasons the great majority of my investigations have been based on living specimens and detailed studies of all forms included here have been made on the living animals with the aid of an oil immersion. Pressure of the cover slip is advantageous since it makes structures more visible. It also slows down the activity of the worm so that more accurate measurements can be completed on the living form. Most of the following observations were made on living cercariae under uniform pressure which was just strong enough to prevent rapid movement.

It would seem that abnormal conditions as yet little understood are at work, when observations show that at least with some species, those leaving the snail soon after it has been placed in a laboratory aquarium, encyst normally, but those appearing later due to an artificial rise in temperature die without encysting. Perhaps their development has been

speeded to such an extent that they break from the sporocyst and emerge from the snail prematurely, death consequently resulting. This may also explain why many hosts remain uninfected during the experimental life history studies of cercariae.

An examination of the data on molluscan infection with the cercariae discussed in this survey brings out a number of interesting features of the work. As previously mentioned, the reporting of many molluscan hosts for the same species of cercaria in the same locality has been questioned. However, Cort (1918) gave several striking examples of the lack of specificity in the choice of intermediate hosts, notably the human schistosome cercariae, *C. douthitti* Cort, 1914, and *C. douglasi* Cort, 1917. He submitted data to support this contention and said: "The data given above seems to clearly indicate that the forked-tailed cercariae readily adapt themselves to new molluscan intermediate hosts."

In fact the very existence of many forms depends upon their cosmopolitan adjustment to various hosts in different localities. This has been proved by my own distributional and experimental studies. In all, emerging cercariae from about one hundred snails were studied. These infected snails represent about 2.98 per cent of the total number collected from all localities.

Descriptions of eight new species presented in this paper were made from material collected in Illinois. This brings the number of described species in the state to a total of thirty-eight. Cort (1914, 1915) was the first worker to describe cercariae from Illinois, and he described these forms more completely also than had been done for any North American larval forms up to that time. Since then other workers, notably Faust (1918, 1918a), Miller (1923, 1926), Beaver (1929) and Horsfall (1930) have published detailed descriptions of one or more Illinois forms. Other references (Faust, 1919; Hopkins, 1933, and Horsfall, 1933) have been made to Illinois larval forms but so far as I am able to determine no other cercariae have been described in enough detail so that their identity may be determined with a fair degree of certainty.

MORPHOLOGY AND CLASSIFICATION

Cercaria sphaerocerca n. sp. (Figs. 1–3).

Enormous, awkward tail distinctive, consisting of three parts, a proximal bell- or funnel-shaped structure into which the body is withdrawn for encystment, a distal portion narrow and elongated, and median, swollen globe many times larger than the body. Body elongate oval, covered with transverse rows of tiny papillae, lacks spines, bears at its anterior end a small stylet without projections; acetabulum posterior to middle of body, larger than oral sucker; six to eight stylet glands on each side of body anterior to acetabulum; pharynx absent; esophagus divides near acetabulum to form intestinal crura which reach slightly posterior to a point midway between ventral sucker and posterior end of body; bladder very elongate, reaches nearly to the acetabulum, surrounded by large,

regularly-placed cells. Measurements taken with body at rest: Length of body 0.470 mm., width, 0.144 mm.; length of distal, narrow part of tail 0.070 mm., length of tail globe 0.362, width of tail globe 0.252 mm., length of bell of tail 56 μ, width 82 μ; total length of cercaria 1.04 mm.; oral sucker 85 μ long and 78 μ wide, longitudinal diameter of acetabulum 0.102 mm., transverse diameter 0.111 mm.

Sporocyst elongate, sac-like, knobbed at narrow end, semi-transparent with sluggish movement, contains many cercariae and germ balls. Cercariae have been found encysted inside the proximal bell of the tail; also found encysted in the cavity of the sporocysts.

This form belongs to the Gorgoderine Cercariae, along with five formerly described species and another new species described below. Its host is *Musculium transversum* from the Salt Fork oxbow at St. Joseph, Illinois. The stylet of this form lacks the lateral points along a ventral sharp blade which have been so well illustrated by Sinitsin (1905) and Lühe (1909) for other members of this group. The large, clear, irregular-shaped divisions in the tail globe are similar to those described for *C. gorgoderae turgescionsis* but the glands bordering the walls of the excretory bladder are like those pictured for *C. gorgoderae pagenstecheri* and not in as regular formation as those of *C. gorgoderae loossi*. The large tail appendage also differs noticeably from that described for any other gorgoderine cercaria.

Cercaria mitocerca n. sp. (Figs. 4–5).

Resembles *C. sphaerocerca* in possessing a long tail consisting of three distinct parts, a proximal, spherical hollow structure where the body is attached, a very long, extremely narrow distal part and a median, swollen portion or globe. Body elongate when extended but has a wide posterior flare when contracted. A median constriction, common to other gorgoderine cercariae, present. Other features are: Numerous papillae over surface of body; spherical acetabulum smaller than oral sucker; esophagus divides midway between oral sucker and acetabulum to form intestinal crura which extend to within 0.13 mm. from posterior end; elongate excretory bladder reaches almost to acetabulum; main collecting tubules extend to anterior end and back to posterior end before they branch; large, spherical bodies fill median part of body posterior to acetabulum; a left, five-lobed and a right, four-lobed structure which are probably testes, and an ovary which is anterior to the left testis. Body is 1.12 mm. by 0.200 mm. when well extended and 0.728 by 0.448 mm. when contracted; tail globe is about 0.448 mm. by 0.185 mm.; narrow thread-like portion 2.13 mm. by 22 μ near base; oral sucker 0.14 mm. by 0.129 and acetabulum 0.126 mm. in diameter.

Sporocysts oval sacs, about 0.98 mm. long and 0.462 mm. wide, often with contracted ends. No independent movement noted even in the younger ones. Encysted cercariae without tails found inside the sporocysts. Cyst wall spherical and transparent.

This species should be placed with the Gorgoderine Cercariae although it differs from all other known forms in that it has no stylet. However, it resembles these cercariae in regard to its long, partially swollen tail, the presence of lateral constrictions on either side of the body just anterior to the ventral sucker, the location of rather prominent

papillae over the surface of the body, the general features of its digestive organs, and the presence of a four-lobed and a five-lobed testis. This cercaria was parasitic in *Actinonaias carinata* in the Sangamon River near Mahomet, Illinois.

Cercaria mesotyphla n. sp. (Figs. 6–8).

Body oval, narrow in front, rounded behind. It has a rapid jerky swimming movement. Spines cover entire body, more dense anteriorly. Other noticeable features of this cercaria are: Tail with prominent fin-fold on posterior half and four groups of longitudinal muscles; acetabulum slightly posterior to middle of body, smaller than oral sucker; short prepharynx, small muscular pharynx about 44 μ wide and 40 μ long, esophagus about 50 to 80 μ in length, intestinal ceca reach almost to posterior end, a sac or pouch at posterior margin of intestinal fork; stylet 23 μ in length without prominent shoulders; numerous penetration glands on either side of body; two germ masses dorsal to ventral sucker; excretory bladder consisting of two vesicles; flame cell pattern of the $2[(3+3+3)+(3+3+3)]$ type. The contracted body is 0.35 mm. long and 0.308 mm. wide, and the tail is 0.308 mm. long. The extended body is 0.476 mm. by 0.196 mm. and the tail is 0.616 mm. long and 0.308 mm. wide near its base. The oral sucker is about 84 μ in diameter and the acetabulum is 60 μ.

Sporocysts long, slender, brown sacs; vary from 5.7 to 3.6 mm. in length by 0.43 to 0.38 mm. in width; no birth pore present.

This xiphidiocercaria belongs to the Cercariae Ornatae and in a subgroup along with *C. hemilophura* Cort, 1914, the only other member of the subgroup. These two cercariae differ from other Cercariae Ornatae in possessing an excretory system of the $2[(3+3+3)+(3+3+3)]$ type. The club-shaped bladder, the absence of a pouch between the ceca, its very short fin-fold and the numerous cystogenous glands of Cort's species help to distinguish it from *C. mesotyphla.* Snails collected from the oxbow at Muncie, Camp Creek at Seymour, East Lake fork near Sadorus and a pond near Rantoul, and identified as *Physa gyrina hildrethiana, Physa gyrina* and *Physa halei* were infected with this species.

Cercaria cystorhysa n. sp. (Figs. 9–10).

Tail not longer than one-half body length when worm is at rest; elongate body with narrow anterior end and subquadrate posterior end; body spines absent; acetabulum from one-half to two-thirds size of oral sucker and posterior to middle of body; stylet 17 μ long with a swollen circular ridge at base of anterior third of stylet, a pair of penetration glands on each side of body, the outer one of which has three lobes, penetration ducts swollen at irregular intervals, crescent-shaped mass of germ cells at the left of and posterior to acetabulum; rectangular bladder with small wrinkles or folds in its walls. Main collecting tubules traced forward to their branches. Extended body 0.14 mm. by 34 μ; tail 0.14 mm. long. Contracted body 73 by 56 μ and the tail 39 μ long. Oral sucker 30 μ long and 27 μ wide; acetabulum 18 μ wide.

Sporocysts grayish or semi-transparent and vary from spherical to oblong, many with knob-like projections at one end. Oblong sacs are 0.195 by 0.121 mm., knobbed ones 0.187 by 0.116 mm. and spherical ones 0.126 by 0.112 mm.

The cercaria belongs to the group of xiphidiocercariae known as Cercariae Microcotylae and in the Cellulosa subgroup. The minute size of

this form, the comparatively small size of the acetabulum, the annulated stylet and the three-lobed outer penetration glands distinguish this form from the other four previously described members of the Cellulosa subgroup. These four species are *C. chlorotica* Diesing, 1850; *C. brunnea* Ercolani, 1850; *C. microcotyla* Filippi, 1854, and *C. pseudornata* Lühe, 1909. *C. cystorhysa* was found in *Goniobasis livescens* from the Salt Fork River at Homer, Illinois.

Cercaria meniscadena n. sp. (Fig. 16).

Tail only slightly shorter than body; visible spines absent; acetabulum posterior to center of body, much smaller than oral sucker; tiny pharynx, beginning of the esophagus, which is the only visible part of the digestive tract; pointed stylet about 17 μ long by 5 μ wide at base, with swollen ridge near its distal end, two large penetration glands on each side of body, the posterior one being characteristically crescent-shaped; small rectangular bladder which becomes U-shaped when body is extended. Contracted body 89 μ by 66 μ and tail 59 μ by 16 μ near base. Extended body is 0.17 mm. long and the tail is 0.15 mm. Oral sucker 33 μ in transverse and 30 μ in longitudinal diameter, acetabulum 20 μ in diameter.

C. meniscadena develops in small, swollen, oblong sporocysts with ends blunt and walls uneven in thickness, nearly colorless. Commonly from two to three cercariae in sporocysts; germ balls also present; sacs have no power of independent movement, at least in the old stages; average sporocyst about 0.185 mm. long and 0.123 mm. wide.

This form also belongs to the Cellulosa subgroup of the Cercariae Microcotylae. Like *C. cystohorysa,* it differs from other previously described members in its minute size, its oral sucker which is considerably larger than the acetabulum and its annulated stylet. It also can be distinguished from *C. cystorhysa* by its bladder which is U-shaped rather than roughly rectangular as in *C. cystorhysa,* by its normally elongated rather than spherical body, by its two pairs of distinctly different penetration glands and by the presence of a pharynx which was not observed in *C. cystorhysa.* Large numbers of the snail, *Pleurocera acuta* in the Sangamon River near Mahomet, Illinois, were parasitized by *C. meniscadena.*

Cercaria acanthocoela n. sp. (Figs. 11–12).

Large body spines in transverse and diagonal rows across the body, very far apart posterior to the acetabulum; small pocket on each side of tail formed by an extension of body over tail's attachment, which bears long setae or spines in its lateral wall; acetabulum smaller than oral sucker, near anterior margin of posterior third of body; short prepharynx, small pharynx about 23 μ long, short esophagus, ceca represented by two narrow strands of tissue reaching back to a point lateral to midline of acetabulum; stylet 30 μ long with a pointed cone constituting its distal third, at the base of which is a thickened hump on either side; seven or eight penetration glands on each side of body which connect with two ducts swollen at various places; two germ cell masses, one posterior to, and one anterior to acetabulum, connected by a narrow strand of cells, excretory bladder consisting of a posterior and two lateral vesicles; four groups of capillaries anterior to acetabulum and two groups posterior to it, with three capillaries in each group.

The contracted body is 0.228 mm. by 0.111 mm. and the tail is 0.117 mm. long. When extended, body is 0.355 mm. by 65 μ and the tail is 0.247 mm. long.

Sporocysts elongate, sac-like structures, vary greatly in size, walls thin and sometimes produced into knobs; average 0.2 mm. to 0.78 mm. long and 15 μ to 0.24 mm. wide. Encysted cercariae found in the liver of same snails, in which the cercariae had developed.

This xiphidiocercaria belongs to the group known as Cercariae Armatae. Of the fifty or more members of this group, it resembles most closely the following five species. The well-developed digestive tract of *C. acanthocoela* distinguishes it from *C. isocotylea* Cort, 1914, and *C. polyadena* Cort, 1914; the latter species also has ten to twelve pairs of penetration glands while the above form has never more than eight pairs. The character of the excretory systems of *C. glandulosa* Faust, 1917; *C. diaphana* Faust, 1917, and *C. indica XVII* Sewell, 1922, the size of the stylets of *C. glandulosa* and *C. indica XVII,* the sucker proportions of *C. diaphana* and the genital cell masses of *C. indica XVII* are all characters which differ considerably from those of *C. acanthocoela.* This species parasitizes *Helisoma trivolvis* in the oxbow at Urbana and St. Joseph and in *H. trivolvis* and *Physa gyrina hildrethiana* in Camp Creek at Seymour, Illinois.

Cercaria tricystica n. sp. (Figs. 13–15).

Body oval in outline and covered with spines anterior to acetabulum. When at rest oral sucker 52 μ in longitudinal diameter and 59 μ in transverse diameter; acetabulum 39 μ in diameter. Other features are: Acetabulum slightly posterior to middle of body; a digestive system consisting of short prepharynx, large pharynx, narrow esophagus, very narrow crura extending to transverse diameter of acetabulum; two lateral groups of penetration glands each consisting of nine glands which connect with bipartite duct; stylet 21 μ long with dorsal ridge similar to that of *C. acanthocoela*; genital mass just posterior to acetabulum; excretory bladder with three vesicles arranged like those of *C. acanthocoela*; narrow caudal pockets which have no spines. When contracted, body is 0.224 mm. by 78 μ and tail is 84 μ long. When well extended body is 0.308 mm. by 56 μ and tail is 0.174 mm. long.

Sporocysts light brown in color, oblong sacs, have thin, elastic walls, about 0.195 mm. long and 85 μ wide.

This cercaria also belongs in the group of xiphidiocercariae known as Cercariae Armatae. It bears close superficial resemblance to only five other forms. Its small tail, sac-like sporocyst, nine pairs of penetration glands and its complete digestive tract readily distinguish it from *C. isocotylea* and *C. polyadena.* Its caudal pockets lack the setae of *C. diaphana,* its stylet is only half as long as, and it has more salivary glands than does *C. diaphana.* Many digestive glands and pronounced caudal pockets with spines distinguish *C. glandulosa* from this new species. The salivary glands of *C. indica XVII* differ in regard to shape, number and the character of their ducts from those of *C. tricystica.* This species was found in *Helisoma lantum* collected at Baton Rouge, Louisiana.

Cercaria cystonchnoides n. sp. (Figs. 17–18).

Cercariae and sporocysts in liver in immense numbers, such swarms of cercariae emerging that they soon make bottom of container white. Cercaria has elongate body with median depression at posterior end of body below tail's attachment. Other outstanding points of structure are: Large stylet 36 μ long and with swollen ridge at base of pointed, anterior end; a group of many penetration glands on each side; oral sucker about 0.101 mm. in diameter, slightly larger acetabulum near center of body and 0.104 mm. in diameter; digestive system consisting of short prepharynx, pharynx about 0.299 mm. long, relatively short esophagus, very narrow ceca which reach almost to posterior margin of excretory bladder; large rectangular or pyriform bladder; eighteen flame cells on each side of body, arranged in six groups of three each; a mass of germ cells posterior to acetabulum. When at rest body about 0.616 mm. long and 0.325 mm. wide through acetabulum, tail is of about same length. Numerous spines cover entire surface, larger in anterior region.

C. cystonchnoides develops in sporocysts which have brownish red pigment, are sac-shaped, have various necks and knobs, majority of them have only two swollen portions. Cercariae were in many different stages of development in cavities of sporocysts.

Two others members of the group to which this cercaria belongs, the Cercariae Armatae, also possess ventral suckers which are larger than the oral suckers. They are *C. tenuispina* Lühe, 1909, and *C. triloba* Filippi, 1857. However, the large size of *C. cystonchnoides,* its very long, homogeneous tail and its characteristic sucker proportions and digestive tract set it apart from the two above-mentioned forms. This new species was found in the snail *Ampullaria depressa,* from Leesburg, Florida.

Cercaria steganocoela n. sp. (Figs. 19–20).

The body shape, stylet, germinal mass and bladder of this form are very similar to those of *C. tricystica* and *C. acanthocoela.* Digestive system resembles that of *C. cystonchnoides* and caudal pockets are like those of *C. acanthocoela.* Body covered with spines in anterior regions; oral and ventral suckers of approximately same size, acetabulum in center of body; two lateral groups of penetration glands, anterior and lateral to acetabulum, with five to six glands in a group; smaller glands are located along the courses of the ducts, which are twisted and swollen at intervals. When contracted, body is 0.224 mm. long and 0.168 mm. wide; when at rest, both tail and body are about 0.28 mm. long; when extended, body is 0.46 mm. by 80 μ. Large, irregular, yellowish sporocysts are in liver tissue of host; length of sacs averages 4.5 mm. and width 0.252 mm.

This cercaria should be placed in the group Cercariae Armatae. Its digestive tract is similar to that of about fourteen other previously described forms of this group, but the number of salivary glands, the shape of the excretory bladder, the presence of large caudal glands and the character of the genital masses enable me to separate it readily from these forms. *C. candelabra* Faust, 1919, resembles most *C. steganocoela* but differs from it in having a distinctly different excretory bladder, a smaller oral sucker, acetabulum and stylet, and in having more numerous penetration glands. The host of this species is *Physa gyrina hildrethiana* collected near Seymour, Sadorus, and Urbana, Illinois.

Cercaria tetradena n. sp. (Figs. 21–22).

Numerous oil-like droplets, bluish in color, and scattered through entire body; tail inserted on the ventral side of body some distance from posterior end, and half as long as the body; stylet lacks pronounced swelling or ridge that is common in the Cercariae Armatae and is 16 μ long; prepharynx, pharynx, esophagus and very long intestinal ceca which reach almost to end of body; four large penetration glands on each side of body which connect to a tripartite duct; unique excretory bladder with large sphincter with thick walls bearing hair-like projections on inner surfaces; a canal leads from sphincter to outside between tail and body; irregular, thick-walled canal extends forward from sphincter and near acetablum widens to form two lateral vesicles. Single genital mass posterior to acetabulum; contracted body is 0.129 mm. by 0.168 mm.; when extended 0.392 mm. by 0.073 mm. and tail is 0.2 mm. long.

Mature sporocysts elongated sacs, orange in color with blunt ends and thin walls.

C. tetradena is another member of the Cercariae Armatae. The unique excretory bladder alone distinguishes it from all other species of the above group. Another form, *C. pandora* Faust, 1921, also has four pairs of penetration glands but its rudimentary intestinal ceca differ radically from the well developed ones of *C. tetradena.* The cercaria was found in the liver of *Helisoma trivolvis* from near Seymour and Henry, Illinois.

Cercaria pteractinota n. sp. (Figs. 23–25).

Body long and narrow; tail nearly three times as long as body when extended. When at rest body 0.27 by 0.104 mm., tail stem 0.532 mm. by 56 μ, and furcae 0.185 mm. by 28 μ at their bases. Prominent features of the cercaria are the following: Numerous spines especially large and thickly set over anterior half of oral sucker; large, conspicuous eye-spots, each containing a small compact mass of pigment in its center, with an open space in front; other prominent pigment masses over body; furcal fin-folds bearing numerous rays or ribs; digestive system consisting of only esophagus and possibly ceca which were not observed; small ventral sucker about 65 μ posterior to oral sucker, with inner surface covered with spines, and with diameter of 29 μ; large oral sucker or penetration organ about 91 μ long and 59 μ wide; numerous penetration glands filling a large portion of the body posterior to the acetabulum; lighter glandular bodies which occupy region ventral to penetration glands; two ventral masses of cells and also two compact bodies above acetabulum which are probably genital primordia. Median excretory tubule extends from small excretory bladder to distal end of tail-stem where it divides, one branch passing into each furca.

Sporocysts are thin-walled elongate sacs, delicate filiform bodies which break easily so that only sections are usually available for study.

C. pteractinota belongs to the Apharyngeal Brevifurcate Distome Cercariae and in Group F along with *C. indica XXXVI* Sewell, 1922, the only other representative of Group F. The possession of numerous pigment masses, of numerous penetration glands, of two germ masses instead of one, and its development in elongated sporocysts, instead of small oval ones separates this new species from *C. indica XXXVI.* It has been found in both *Helisoma trivolvis* and *Physa gyrina* collected

near Paris, Illinois, and in *Helisoma lantum* from Baton Rouge, Louisiana.

Cercaria louisiana n. sp. (Figs. 26–27).

Assumes a hook-like appearance when hanging in the water of container. Body as narrow as tail-stem and normally a little shorter than stem; tail-stem and furcae of about the same length; crown of large, narrow spines over anterior third of oral sucker; absence of caudal bodies in tail-stem; narrow fin-fold on each furca; several fine striations in furcae; absence of a digestive system, with the exception of cavity of oral sucker; two dorsal, pigmented eye-spots just in front of anterior penetration glands; six large penetration glands in posterior region of body, which connect with bipartite ducts that extend anteriad; germinal cell-mass posterior to penetration glands. When the cercaria is resting or quiescent the body is 0.168 mm. by 43 μ, tail stem is 0.224 mm. by 33 μ and furcae are 0.224 mm. by 17 μ at bases.

C. louisiana is a representative of the Apharyngeal Longifurcate Monostome Cercariae. Two other representatives of this group are known, namely, *C. indica XXVII* Sewell, 1922, and *C. sewelli* Faust, 1926. *C. louisiana* lacks the rhabdocoele gut and numerous penetration glands of *C. indica XXVII* and possesses pigmented eye-spots and furcal fin-folds, both of which *C. indica XXVII* lacks. *C. sewelli* has only two pairs of penetration glands and a dorsal body keel, characters which separate it from *C. louisiana*. *Physa gyrina* from near Baton Rouge, Louisiana, harbored this cercaria.

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Explanation of Plates

All figures were made from living material with the aid of a camera lucida. In all cases the penetration glands were drawn after neutral red had been added. Internal structures were in many cases inserted free hand. The scales for Figs. 3, 8, 10, 12, 14, 15, 17, 20 and 22 represent 0.01 mm.; for Figs. 1, 9, 11, 13, 16, 19, 21, 23, 24 and 26 it represents 0.05 mm.; for Figs. 2, 4, 5, 6, 7, 18, 25 and 27 it represents 0.1 mm.

Figs. 1–3. *C. sphaerocerca.* Drawings showing respectively the ventral view of the body; the encysted form in the tail globe; the side view of the stylet.

Figs. 4–5. *C. mitocerca.* Ventral view of body; drawing showing the tail globe with its attached filiform appendage.

Figs. 6–8. *C. mesotyphla.* Ventral view of the body; excretory system from a ventral view; dorsal view of stylet showing globular base.

Figs. 9–10. *C. cystorhysa.* Ventral view of contracted body; dorsal view of stylet to show lateral shoulders.

Figs. 11–12. *C. acanthocoela.* Ventral view of body; dorsal view of stylet.

Figs. 13–15. *C. tricystica.* Dorsal view of body; dorsal and lateral views of stylet.

Fig. 16. *C. meniscadena.* Ventral view of cercaria.

Figs. 17–18. *C. cystonchnoides.* Dorsal view of stylet; same view of body showing excretory system.

Figs. 19–20. *C. steganocoela.* Dorsal view of body; lateral view of stylet.

Figs. 21–22. *C. tetradena.* Dorsal view of body; same view of stylet.

Figs. 23–25. *C. pteractinota.* Furca showing the rays of fin-fold; dorsal view of anterior end; same view of entire cercaria.

Figs. 26–27. *C. louisiana.* Dorsal view of body; outline drawing to show relation of parts of body.

PLATE I

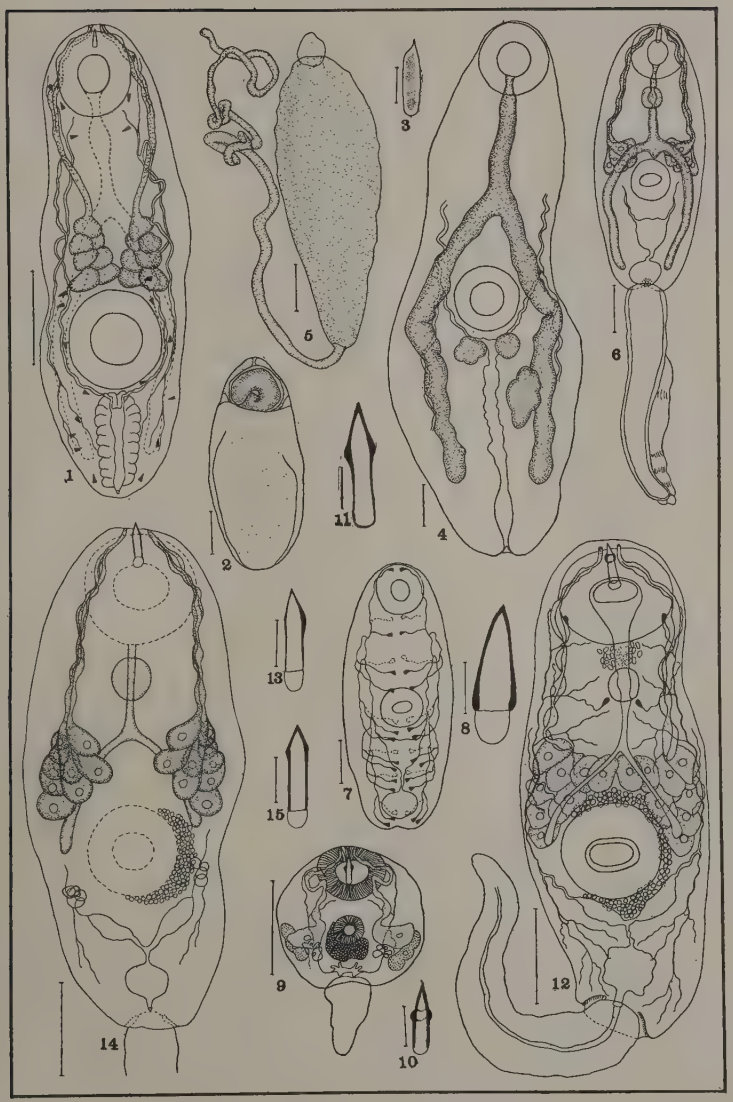
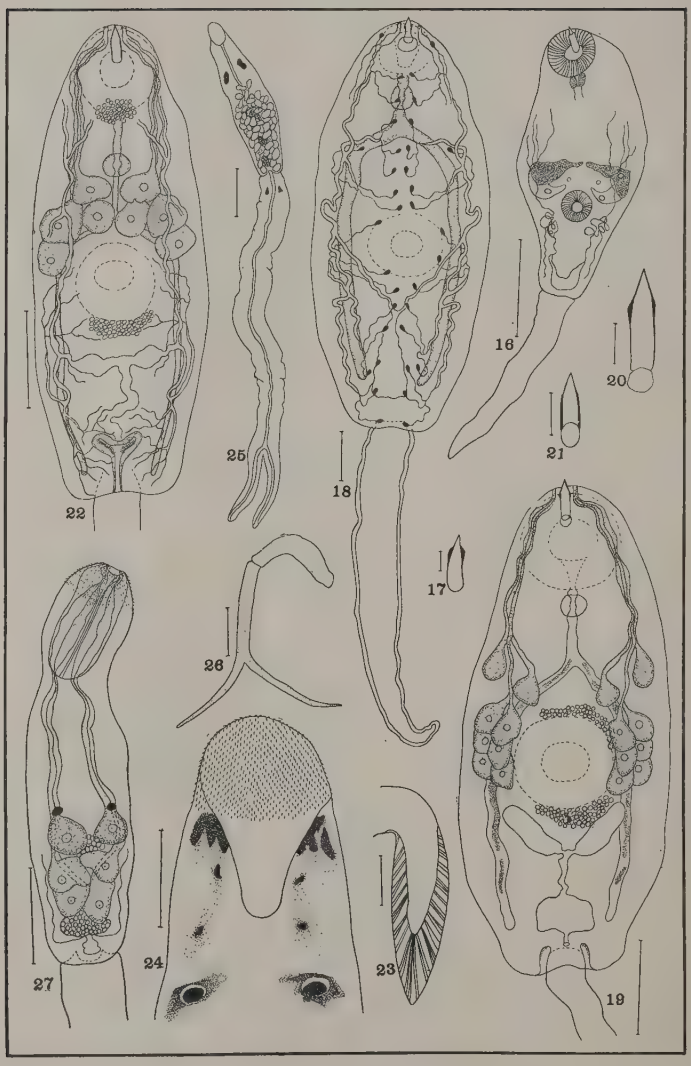


PLATE II



THE COMPARATIVE MORPHOLOGY OF THE SPOROZOITES OF THE HUMAN SPECIES OF *PLASMODIUM*, MARCHIAFAVA AND CELLI, 1885[1]

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While the general features of the morphology of the sporozoites of *Plasmodium vivax* and *Plasmodium falciparum* are well known, we are not aware that the sporozoites of *Plasmodium malariae* have been figured. The material we have available made it appear desirable to fill this lacuna.

The sporozoites figured in Plates I, II, and III were traced from camera lucida drawings. Those in Plates I and III were made from smears prepared from the salivary glands of specimens of *Anopheles quadrimaculatus* in which infections had just matured. Glands were crushed in a small drop of albumin water, spread and fixed in hot sublimate alcohol. They were then stained, either with iron hematoxylin or with Giemsa's stain. Those in Plate II were from glands which had been crushed in saline, air dried, fixed in the methyl alcohol of Wright's stain, and stained with the latter.

We have never observed any motility of sporozoites, in the sense of locomotion, in unfixed fluid preparations made from the salivary glands. In unfixed fluid preparations of stomachs, in which sporozoites were escaping from mature cysts, a very active vibratory movement of groups or masses of sporozoites is noted, but we have not been able to distinguish the source of the motion. It has not been possible to discern the contribution made by any of the individuals comprising the group. In some of these preparations, there has been a suggestion that sporozoites isolated in the fluid were progressing by a very slow gliding movement. Most sporozoites in gland preparations finally undergo an imperceptible flexation without any change of location. This is probably a degenerative change.

Sporozoites are long slender organisms, with a length from 10 to 30 times their diameter. In general their diameter is uniform, but some individuals appear to be enlarged in the middle portion, while others have a greater diameter toward one extremity. The length is subject to great variation, although the limits of variation among those secured

[1] The studies and observations on which this paper is based were conducted with the support of and under the auspices of the International Health Division of The Rockefeller Foundation, in cooperation with the Florida State Board of Health.

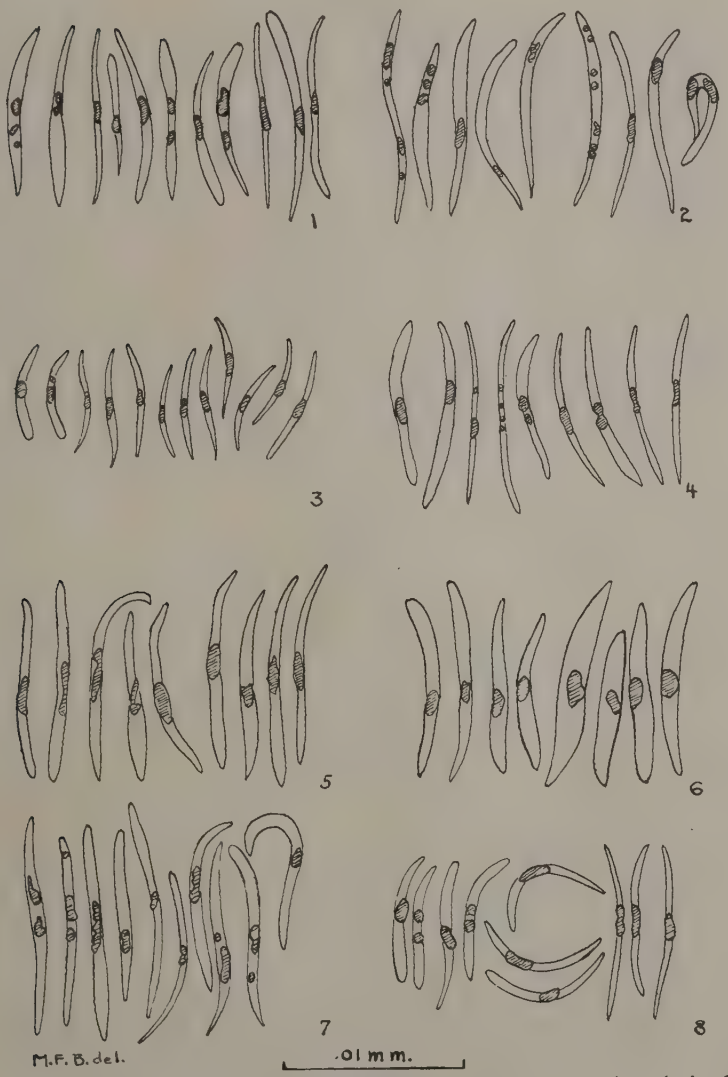
from any particular mosquito will not be great. Extreme variations in length may lie between 6 and 15 micra. The diameter is likewise subject to considerable variation. The extremities may be similar or dissimilar. They may both be blunt, both finely pointed, or one may be blunt and the other pointed. The latter form appears most frequently with *P. vivax,* while with *P. falciparum* both ends tend to be pointed. In general, sporozoites of *P. falciparum* are the finest, those of *P. malariae* the coarsest. With Giemsa's or Wright's stain there is demonstrable a central, usually elongate, mass of reddish chromatin. Its diameter may appear greater than that of the cytoplasm, causing a bulging. Rarely, two masses are observed in juxtaposition. If the chromatin does not exactly occupy a central position, it is usually displaced toward the pointed extremity. Rarely, the mass is irregular and shrunken, or three to four small, round, discrete, masses are observed. The density of the chromatin appears to be greatest in *falciparum,* and most diffuse in *malariae.*

Vivax sporozoites, when stained with iron hematoxylin and decolorized, usually show a single black-staining mass occupying the same position as does the red material seen in polychrome stained preparations. On the other hand *falciparum* sporozoites stained with iron hematoxylin show a series of three or more small, spherical, black masses usually lying toward either extremity, with the central portion vacant. Some (III, 3) show irregular diffuse masses of gray material.

Sporozoites of any species vary within such wide limits that attempts at identification of species on morphological grounds would be unreliable.

All figures in plates I–III are traced from camera lucida drawings.

PLATE I

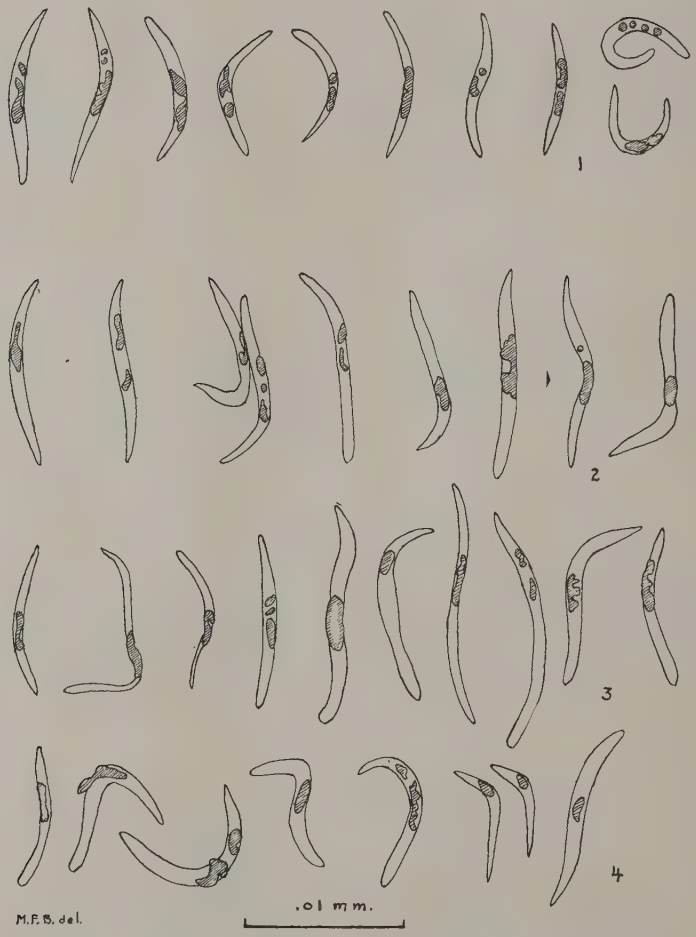


Plasmodium vivax. All members of each group of sporozoites derived from the salivary glands of the same mosquito.

Groups 1, 2, 3, 4. Gland crushed, fixed in sublimate alcohol, and stained with iron hematoxylin stain.

Groups 5, 6, 7, 8. Gland crushed, fixed in sublimate alcohol, and stained with Giemsa's stain.

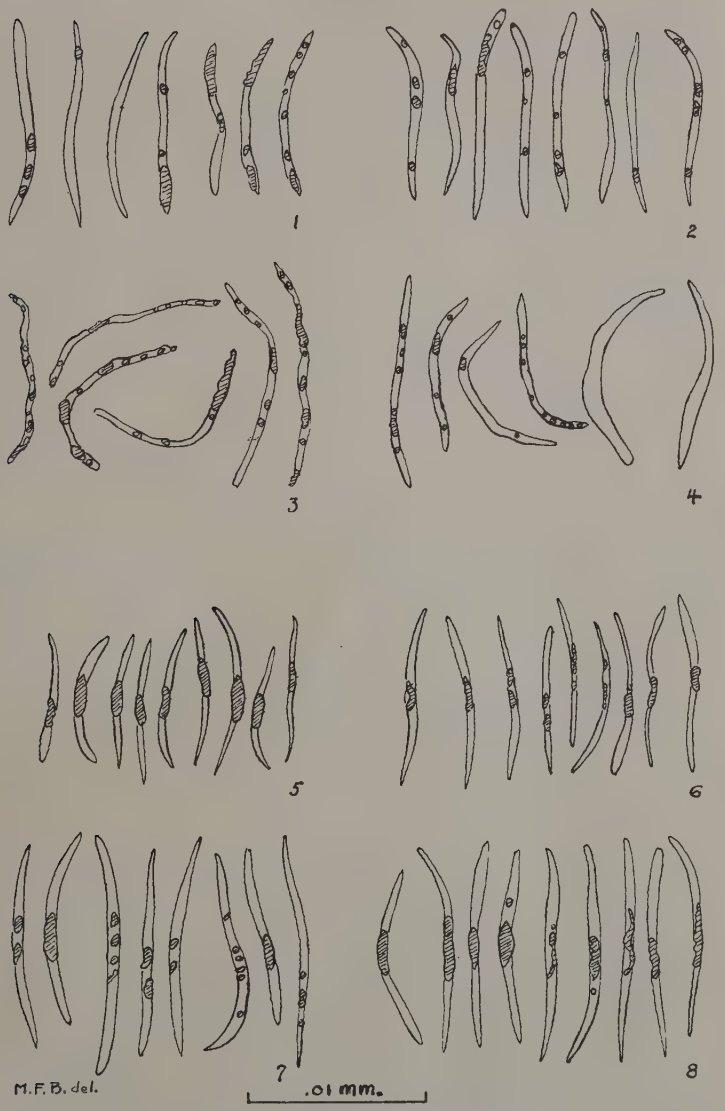
PLATE II



Plasmodium malariae. Sporozoites of groups 1 and 2 derived from the glands of two different mosquitoes. The sporozoites represented in groups 3 and 4 were selected from gland smears from several different mosquitoes.

Groups 1, 2, 3, 4. Glands crushed in saline, air dried, fixed with methyl alcohol, and stained with Wright's stain.

PLATE III



Plasmodium falciparum. All members of each group of sporozoites derived from the salivary glands of the same mosquito.

Groups 1, 2, 3, 4. Gland crushed, fixed in sublimate alcohol, and stained with iron hematoxylin stain.

Groups 5, 6, 7, 8. Gland crushed, fixed in sublimate alcohol, and stained with Giemsa's stain.

NOTES ON THE SPECIES OF *TRIAENOPHORUS* IN CANADA

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The published records on *Triaenophorus* from Canadian fishes have been few and contradictory, although this cestode is perhaps one of the most common and widely distributed fish parasites known. The data upon which the present study is based were obtained from the examination of a great number of triaenophorid cestodes from the pike (*Esox lucius*) and from coregonid fishes of western and eastern Canada. The purpose of this paper is to point out the outstanding specific features by which this cestode is characterized.

Two distinct species of *Triaenophorus* commonly occur in Europe, *Triaenophorus nodulosus* Pallas (1760) (= *T. tricuspidatus* Bloch, 1779) and *Triaenophorus crassus* Forel (1880) (= *T. robustus* Olsson, 1893).

Triaenophorus nodulosus Pallas (1760).—The adult stage occurs chiefly in the intestine of the pike, while the plerocercoid stage has been found in the visceral organs, especially the liver, of over thirty different species of fresh-, brackish- and salt-water fishes. Young pike have also been reported as intermediate hosts of *T. nodulosus*. The length of the European specimens examined varies between 100 and 300 mm. The anterior portion of the body, immediately following the scolex is smooth and filamentous for some distance. The strobila is flat, the margins being thinner than the median portion. The scolex is small and narrow, with the terminal disc usually inconspicuous, and the bothria shallow and not sharply outlined. The hooks are small, with a shallow and remarkably curved basal plate bearing four projections. The marginal ones are long, slender and deeply curved, while the two median projections are somewhat shorter and the one which is directed backward is not as pointed as the one which is directed outward. The scolex is not sharply delimited from the strobila, the latter continuing as a thin filament for some distance before it is thrown into wrinkles, which are somewhat finer than in *T. crassus*.

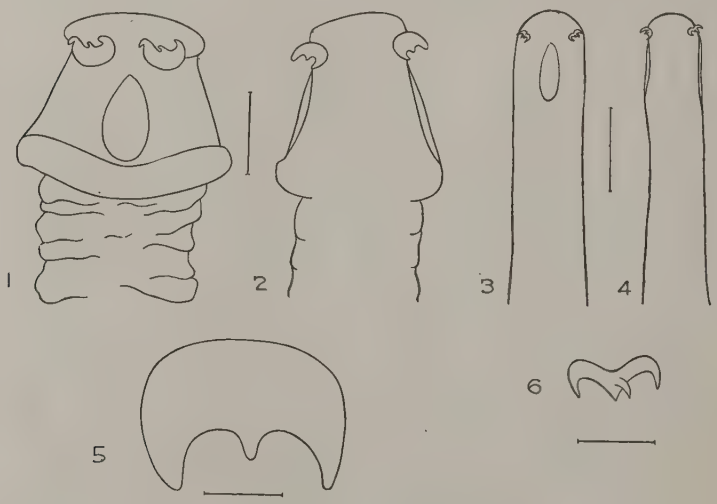
Triaenophorus crassus Forel (1880).—The adult stage of this species is also an intestinal parasite of the pike, while the plerocercoid occurs in the muscles of coregonid and salmonid fishes. The species was first described by Forel (1880) in Switzerland, but his work was overlooked until Fuhrmann (1926) rediscovered it. (*Vide* Scheuring, 1929). Olsson (1893) described the same species as *T. robustus*. Scheuring (1921) reported that *T. crassus* is not uncommon in the larger lakes of

Germany. *T. crassus* has also been reported from Sweden and Finland by several authors. In general appearance this species is larger and thicker than *T. nodulosus*. It reaches a length up to 650 mm. The strobila is thrown into coarse wrinkles which start immediately behind the scolex. The strobila is thick, especially at the margins, so that a transverse section appears more or less rectangular. The scolex is blunt and large, usually sharply delimited from the strobila by a collar-like thickening. The bothria are sharply outlined and deeper than those of *T. nodulosus*. The terminal disc is conspicuous and more or less rectangular in shape. The hooks are blunt, being composed of a deep basal plate with three projections, which are short and very slightly curved. The marginal points may vary in length, one sometimes being shorter than the other, in which case the median projection is always the shortest. The fourth projection, which appears in *T. nodulosus,* is hardly noticeable.

The genus was first reported for this continent by Cooper (1918) and Hjortland (1927) subsequently reported "*T. robustus*" from Minnesota. The occurrence of *Triaenophorus* in Manitoba was first reported by Nicholson (1928). He (1932) also studied the pathogenicity of the larva, finding it to be non-pathogenic to man. Newton (1932) discussed the biology of "*T. tricuspidatus*" in western Canada. Wardle (1932) accepted his identification and, evidently without reference to European specimens, united the two distinct species *T. crassus* and *T. nodulosus* into one species with two forms, "*T. tricuspidatus*" morpha *microdentatus* and morpha *megadentatus*.

Wardle apparently overlooked the fact that the hooks are movable and that after fixation one finds them at different angles in relation to the longitudinal axis of the parasite, which may give the impression of a deeper or shallower basal plate when the hooks are *in situ*. The author has isolated and compared the hooks from about one hundred specimens, but in no case was a hook found with a plate twice as deep as another of the same width. All basal plates of the same width were found to have approximately the same depth. In the drawings given by Wardle (1932, p. 13, fig. 5) he shows two hooks having basal plates of the same width but with one appearing twice as deep as the other. Careful examination of many isolated plates leads to the conclusion that these two apparent types are only different views of the same hook. The blunt appearance of the hooks is characteristic of all the specimens studied, although several lesser variations may appear, *e.g.*, one of the marginal projections may be shorter than the opposite one, the corners of the basal plate may be sharper or more rounded, and the width of the basal plate itself may vary within small limits. According to Scheuring the width of the basal plate, the height of the marginal

projections taken from the tip to the distal margin of the plate, and the depth of the basal plate vary from 90/45/23 μ to 140/75/45 μ in *T. nodulosus,* while in *T. crassus* the same measurements vary from 285/240/125 μ to 300/255/135 μ. The average measurements for Canadian material are 275/245/138 μ. The widest basal plate found was 290 μ and the deepest one measured 163 μ. Measurements of many hooks seemed to indicate that the narrower plates are relatively somewhat deeper, as 260/255/152 μ. The depth of the plate varies within very small limits, but in general it does not vary as greatly in my material as is indicated by Wardle. The general shape of the scolex and strobila in Canadian specimens is identical with that of European *T. crassus.* The larvae from coregonid fishes closely resemble the adults from *Esox lucius.*



Explanation of Figures

Figure 1. Scolex of *Triaenophorus crassus,* surface view.
Figure 2. Scolex of *Triaenophorus crassus,* lateral view.
Figure 3. Scolex of *Triaenophorus nodulosus,* surface view.
Figure 4. Scolex of *Triaenophorus nodulosus,* lateral view.
Figure 5. Isolated hook of *Triaenophorus crassus.*
Figure 6. Isolated hook of *Triaenophorus nodulosus.*
The lines in figures 1–4 are 0.5 mm. long, those in figures 5 and 6, 0.1 mm. long. The drawings were made with the aid of a camera lucida.

According to Wardle (1932) the nodular appearance of the strobila and the ring-like thickening of the posterior border of the scolex are artefacts induced by exposure to unnatural media. My examinations of

living specimens under a variety of conditions indicate that the collar-like thickening is almost a constant anatomical character.

It is remarkable that Newton (1932) described the cysts as being provided with a "cauda," while others and the present author have found the encysted larva, not the cyst, to be provided with such an appendage.

T. crassus seems to have more of a northern distribution in this continent, as was pointed out by Fuhrmann (1910) for the European *T. crassus*.

From the above observations the conclusion can be safely drawn that the common type of *Triaenophorus* in Canada, the adult of which is the intestinal parasite of *Esox lucius* and the larval stage of which occurs in the muscles of coregonid fishes, is *Triaenophorus crassus*. The occurrence of *T. nodulosus* is rare. The adult has been found in a few cases in the intestine of *Esox lucius,* the plerocercoid encysted in the liver of *Perca flavescens*.

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SPECIES OF CALLIPHORIDAE CONCERNED IN THE PRODUCTION OF MYIASIS IN DOMESTIC ANIMALS, MENARD COUNTY, TEXAS

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It is common knowledge that *Cochliomyia* species,[1] the so-called Texas screw-worm flies, are the most important species concerned in the production of myiasis in domestic animals in the Southwest, but what other flies are also concerned in the production of wound myiasis in this area and the extent of their injuries to livestock are questions which have never been definitely determined. In an attempt to answer these questions, certain observations were made during 1931, 1932, and 1933, at Menard, Tex. The present paper is a report of the results.

METHODS AND MATERIALS

Sheep, goats, calves, and grown cattle infested with maggots were placed in stalls provided with especially designed floors which received and trapped the larvae as they dropped from the wounds of the host.

In these experiments some of the infested animals used were brought in from the range and hence the duration of infestation was not known. Others were wounded animals which became infested in pens, the time of attack being definitely known. The infested animals were kept in the best physical condition possible and were not placed in the collecting pens until the larvae in the wound were nearly ready to leave the host.

Twice a day all the larvae and pupae were removed from the trap pans and placed on slightly moist sand in pint Mason jars. The specimens collected during 1931 and 1932 were left in the Mason jars until all living pupae had produced adults. During 1933 the larvae were placed in Mason jars and allowed to pupate and then the pupae were removed and placed in small screen cages. These cages were placed over water in an airtight container, which provided a relative humidity of approximately 100 per cent. This technique, which was suggested by Roy Melvin, of the Bureau of Entomology and Plant Quarantine, yielded a considerably higher percentage of adults as compared with that used

[1] It has been shown recently by Emory C. Cushing and W. S. Patton (Ann. Trop. Med. and Parasit., vol. 27, no. 4, pp. 539–551, December 20, 1933) that heretofore two species have been confused under the name *Cochliomyia macellaria* Fab. The two species were not differentiated in these experiments.

during the seasons of 1931 and 1932. As soon as adults appeared in the jars or cages they were removed and identified.

During the period August 29 to October 14, 1931, 11,865 larvae were collected from wounds in sheep and goats; 5,697 of these were mechanically removed from wounds, and 6,168 were collected after the larvae had voluntarily dropped from the wounds of animals. In 1932, 11,041 larvae were collected at more or less regular intervals during the period May 30 to September 29. Of these, 862 grown larvae were mechanically removed from wounds, and 10,179 were collected after having normally dropped. In 1933, 31,555 larvae, all of which dropped normally from the hosts, were collected at rather regular intervals during the period March 21 to July 13.

The total number of adults reared from all larvae collected during the three years was 46,242.

EXPERIMENTAL DATA

The data presented in table 1 show the date of collection during the three years in question, the number of larvae collected each month, the species reared, and the number of adults of each species produced.

TABLE 1.—*Flies reared from wounds of cattle, sheep, and goats infested in nature. Menard County, Texas.*

Date collected	Number of larvae collected	Number of adults reared	Per cent of*	
			Cochliomyia species	Phormia regina
March (1933)	258	249	0	100
April (1933)	1,100	943	19	81
May (1932–1933)	3,018	2,608	43	57
June (1932–1933)	26,256	24,034	99	1
July (1932–1933)	5,804	4,765	99.7	0.3
Aug. (1931–1932)	4,739	2,882	99.9	0.1
Sept. (1931–1932)	8,468	6,738	99.8	0.2
Oct. (1931)	4,818	4,023	100	0
Total	54,461	46,242	94	6

* In addition, one specimen of *Sarcophaga* sp. was reared in August and one in September, 1931; and two *Sarcophaga plinthopyga* were reared in August, 1932.

DISCUSSION

The observations recorded here show that during 1931, when all collections were made during the late summer and fall, 99.82 per cent of all adults reared consisted of *Cochliomyia* spp., and only 0.16 per cent consisted of *Phormia regina* and 0.02 per cent of *Sarcophaga* sp. Almost identical results were obtained in 1932, when collections of larvae were made from May 30 through September: 99.85 per cent of all the adults reared during that year consisted of *Cochliomyia* spp., while *P. regina* constituted only 0.12 per cent, and *Sarcophaga plinthopyga* 0.02 per cent of the total.

Nearly 58 per cent of all the larvae collected for this study were taken during the period March 21 to July 13, 1933. As a result of the early collections of this year, the importance of *P. regina* as a producer of wound myiasis in domestic animals was very clearly demonstrated. Even though the population of *P. regina,* which is a cold-weather species of blowfly and very abundant during early spring, decreases to the point where by the last of May it rarely composes more than 2 to 3 per cent of the total of all blowflies present in the area in question, it, nevertheless, composed over 67 per cent of all of the adults reared during the period March 21 to May 26, inclusive. Furthermore, it is of even greater significance to note that larvae of this species only were collected from wounds up to April 17, and that it was still present in greater numbers than *Cochliomyia* spp., in wounds containing mixed infestations, as late as May 14. These observations appear to indicate clearly that *P. regina* is a well adapted facultative sarcobiot that is capable of causing wound myiasis and serious injuries to domestic animals under the environments encountered in nature in the area in question.

SUMMARY

1. Three species of Calliphoridae were reared from wounds of domestic animals infested in nature in the area studied.

2. *Cochliomyia* spp., the screw-worm flies, composed over 99 per cent of the total of all adults reared from larvae found in wounds of domestic animals during the summer and fall months.

3. *Phormia regina* Meig., the black blowfly, constituted over 67 per cent of all adults reared from larvae developed in wounds of domestic animals during the spring months in 1933.

4. *Sarcophaga plinthopyga* Wied., a flesh fly, was found in very limited numbers in wounds of domestic animals.

THE EFFECT OF PARASITISM ON GROWTH OF PERCH IN THE TROUT LAKE REGION[1]

S. X. Cross

In the summers of 1931, 1932, and 1933 a survey of the parasites of fish of the Trout Lake region of northern Wisconsin was undertaken as a part of the biological program of the Wisconsin Geological and Natural History Survey. During the past eight summers the Survey has carried on a regular program of research at the station established at Trout Lake in 1925. This work is connected intimately with the work carried on for many years on the lakes of Wisconsin by Dr. E. A. Birge and Dr. C. Juday. Since the beginning of their program a large amount of informatiion on the chemistry and biology of the lakes, has been collected. As a great deal of data had been acquired on the conditions surrounding the fish life of the region, a study of the parasites of fish was included in the program in 1930. In 1930, Dr. C. A. Herrick made a preliminary study of the fish parasites of the region. He collected most of his material by placing the entire viscera, of specimens to be studied, in alcohol for parasite counts to be made during the winter. This was necessitated by the large amount of material to be examined and by the fact that the purpose of the study was primarily to discover the amount of parasitism present rather than to study any of the specific forms.

With this as a background the present study was undertaken in the summer of 1931. It has been made as nearly quantitative as possible in view of the large amount of material collected. Each host was considered separately with regard to the number of each species of parasite harbored. Such parasites as the larvae of the cestode *Triaenopherous nodulosus* or *Myxosporidia* form cysts of varying sizes in the viscera or other parts of the body, and in these cases note was taken of the relative mass of the parasite tissue present.

Most of the perch examined were specimens also used in growth studies.[2] In this work the fish were individually marked with serial numbers corresponding to the number given the scale sample. This made it possible later to know the length, weight, and age of each individual.

METHODS

The fish used were taken by gill nets or hook and line fishing. Fish were taken with hook and line only when specimens were desired from

[1] Notes from the Limnological Laboratory of the Wisconsin Geological and Natural History Survey. No. 54.

[2] Growth studies made and scales read by Dr. Edward Schneberger.

water where it was impractical to use a net or when net specimens were too few to keep the laboratory supplied. The reason for using nets in preference to hook and line fishing was the generally accepted belief that fish taken in this way make a more representative sample of the population of the water fished. The fish were measured (standard measure), weighed, and a scale sample taken from each at the laboratory by those working on growth studies. Each scale envelope was numbered and for each there was a paper slip also bearing the same number. This numbered slip was placed in the mouth of the fish after the scale sample was taken.

In examining the specimens in the laboratory each fish was first examined for external parasites and then for those in the flesh and skin. The mouth and gills were next examined; the latter were removed and examined, with the aid of a dissecting microscope, for the presence of copepods or myxosporidia. The entire intestine was opened and floated out in a Petri dish for examination with the dissecting microscope. All parasites saved for further study were fixed or preserved in 70 per cent alcohol while still living. The copepods were placed directly in alcohol. Cestodes, trematodes, and nematodes were all fixed before being stored in alcohol.

PRESENTATION AND DISCUSSION OF DATA

The fish represented in table 1 were arranged in age groups for a comparison of growth of those infested heavily with those infested lightly. These groups are made up of all the fish having the same number of annular rings on their scales.

It is impossible to set any sharp limits for the numbers of the various parasites above which one may say a fish is heavily infested or below which its infestation is light or medium. This would be quite simple if the fish were all parasitized with but one species of parasite and all of the fish were about the same size. When we consider the actual situation and must deal with fish harboring a number of different species of parasites the problem of dividing them as to intensity of infestation is more complex. Some individuals harbor a number of large cestode cysts in the viscera with few other parasites. Others may have only encysted trematodes in the flesh or adults in the intestine, and still others may have some of all the species of parasites found in that species of fish in the vicinity. If then one sets out to group these infestations into heavy, medium, and light, he must in every case consider the parasites present with regard to their size and numbers and the evident pathology seen on macroscopic examination. This was done in the preparation of table 1, and an attempt was made to group those fish together that were seemingly about equally handicapped by the parasites they harbored when examined.

TABLE I.—*Size variation in perch with different parasite burdens*

Year	Age of fish	Number of fish	Heavy infestations		Medium infestations		Light infestations		Per cent of difference in heavy and light infestations	
			Length in mm.	Weight in gms.	Length in mm.	Weight in gms.	Length in mm.	Weight in gms.	Length in mm.	Weight in gms.
Silver Lake										
1931	4	41	113	23	121	27	126	33	12	43
1931	5	13	146	48	162	64	172	93	18	94
1932	4	22	124	29	147	54	149	55	20	89
1932	5	35	116	27	129	36	146	55	26	104
Average 1931–32 .	4–5		125	32	140	45	147	59	19	75
Muskellunge Lake										
1931	4	86	104	18	112	23	118	25	13	39
1931	5	40	124	27	133	39	154	54	24	100
Average 1931	4–5		114	22	122	31	136	39	18	70
Nebish Lake										
1931	2	23	118	28	128	32	139	46	18	64
1931	3	38	142	48	150	60	158	67	11	39
1932	2	22	121	29	122	30	136	42	12	45
1932	3	85	144	50	165	72	163	70	13	40
1932	4	46	147	50	179	92	187	110	27	120
Average 1931–32 .	2–3–4		134	41	149	57	156	57	16	61
							Average difference for all fish		18	67

Those fish that contained only very few parasites or none at all were placed in the column headed "light infestations"; those under the heading "heavy infestations" had intestines, body cavity, or flesh heavily infested or had large numbers of parasites in the intestine and the rest of the body as well; while those called "medium infestations" had parasites in fewer numbers but were well infested in one or all of these regions. Allowance was made in all cases for the relative size of the parasites so that a fish containing a large *Triaenophorus nodulosus* cyst in its viscera was considered more heavily infested than one harboring several small trematodes in the intestine or fewer than 100 strigeid cysts in the flesh. From the figures obtained for perch it made little difference where the parasites were located in the body of the host. Trematode or cestode cysts in the flesh, when numerous, had as much effect as adult parasites in the intestine. The majority of the parasites of these perch were encysted in the flesh or the viscera.

The fish considered in table 1 represent only a small per cent of the perch studied and only a portion of those from three lakes. This is due to the fact that the ages of the other species of fish are not yet available and also to the fact that there are too few fish in the age groups, not considered here, to make tables that would be indicative of the general condition. Only those perch were listed of which there were sufficient numbers in each age group to give some idea of the situation among fish in the lakes studied.

Table 1 is a summary of 11 tables made from the records of the individual fish for each group indicated in the column "age." Only those fish are included in this table for which the individual age, as read from the scales, is known. The figures given for ages represent the number of annular rings found on the scales. Thus, a fish marked in this table as age 4 is one with four annular rings on its scales, showing that it has passed through four winters and was in its fifth summer when caught. For the age groups younger and older than those given, no tables were made because there were too few fish examined for any one age group of those ages. Each group represented in the table was composed of the number of fish indicated in the column "number of fish" and the figures given are averages for the weights and lengths of the fish in the respective groups. The right-hand column of the table shows the percentage of differences in weight and length between the heavily infested and the lightly infested fish of the group.

From the Survey's records of the three lakes represented in the table it is evident that the fish population is most crowded, for both food and space, in Muskellunge Lake, and least in Nebish Lake. Silver Lake has about the same population problems as those of Muskellunge Lake.

In the figures given for Nebish, the most lightly populated of these

lakes, two things are evident. These are (1) that the fish are larger for their age than those in the other two lakes and (2) that there is generally less difference between the heavily and lightly infested fish in this lake than in either of the others. In Nebish, the infestations were just as heavy as those in the other two lakes but the differences noted may easily be due to the number of fish occurring in the lake and the greater abundance of food for the fish there. Silver and Muskellunge Lakes are very similar in respect to crowding and both have a greater variety of species of fish than has Nebish Lake. The former lakes are well stocked with the larger game fish as well as with large suckers while the population of the latter lake is limited to perch, rock bass, black bass, and various small minnows.

Another interesting feature of Table 1 appears in the column on the extreme right headed "Per cent of difference in heavy and light infestations." This column represents the percentage of difference in weight and length of the average fish with light infestations over the average heavily infested fish. The greatest difference in weight and length is shown in the case of four-year-old perch from Nebish Lake (Table 1). Here the lightly infested fish are 27 per cent longer and 120 per cent heavier than their heavily infested fellows of the same age. The least difference seen in any of these lakes also occurs in the records of Nebish Lake. This is shown in the three-year-old group for 1931 where the difference in length is only 11 per cent and in weight 39 per cent. In every case demonstrated in Table 1 the fish with light infestations are more than 10 per cent longer and more than one-third heavier than those with heavy infestations.

Another thing brought out by a study of Table 1 is that in every case, with the exception of fish from Nebish Lake for 1931, the older fish show more difference in size between heavy and light infestations than is shown in those fish one year younger, *i.e.,* for four-year-old perch from Silver Lake in 1931 there is a difference in length of 12 per cent and a difference in weight of 43 per cent while the five-year olds show differences of 18 per cent in length and 94 per cent in weight.

It is hard to give a satisfactory explanation for this phenomenon but it may help to explain several things, the most important of which is the occurrence of slow growing individuals observed in most species of the fish so far reported. Among all the fish for which growth studies have been made, some individuals have been found that were small for their age and showed from a study of their scale rings that they had grown slowly during most of their lives. This would suggest the possibility that fish that become heavily infested early in life are handicapped from the time they are very young and start out in poor condition each spring.

Being inferior in condition, perhaps of inferior strength and speed, it is quite possible that they may each year be forced to feed in out-of-the-way places where parasites are more abundant, due to the presence of larger numbers of infested intermediate hosts and other heavily infested fish like themselves.

If this is their fate they may well have continued heavily parasitized during their entire growth period. This is of course purely theory as the data are not yet sufficient to be conclusive. If, however, this is the case, the older age groups should have more fish showing this accumulative effect of heavy parasitism and would, therefore, show a greater difference in size between the heavily and lightly infested individuals.

This would seem, at first, to be contradictory to a later observation in this paper, namely that among the older fish no such differences were seen that could be correlated with parasitic infestations. This may be due to the fact that most of the fish, 7 years old or older for perch, are usually very lightly infected, particularly with internal parasites. Most of the specimens of this age had very light infestations when they had any at all. Although this may not be a general rule for fish it is not an uncommon phenomenon in other phyla of the animal kingdom. Herrick (1925) has demonstrated that there is a definite age immunity factor in the case of chickens infected with *Ascaridia perspicillum*. In 1928 he also demonstrated the existence of this factor in the case of dogs with relation to their parasite *Ancylostoma caninum*. In both of these studies it was shown that animals that are fully mature have a strong resistance to infection with parasites that infect them easily when young. In the case of fish this may well be due to changing food habits as the fish grows larger. Age resistance may also prove to be an important factor in the case of fish when larger numbers of old fish have been studied.

A discrepancy in size is seen in the records of the perch from Silver Lake for 1932. In this case the five-year-old fish average smaller than the four-year-old fish. This does not seem to be correlated in any way with the parasites found.

Table 1 shows that the fish with light infestations average from 11 to 27 per cent longer and 39 to 120 per cent heavier than those with heavy infestations of parasites. This is an average of 18 mm. longer and 67 gms. heavier for those fish that were only lightly infested or negative as for all fish, regardless of age, as shown in the bottom line, shows those compared with those having heavy parasite infestations. The average with light infestations are shown to be 18 per cent longer and 67 per cent heavier than those with heavy infestations.

This effect is seen most clearly among fish that are several years old but not old enough to have slowed up much in growth. Although the

figures for older perch are too few to use for conclusive comparison, those few obtained show little variation in size that can be correlated with parasitic infection found when the fish was caught.

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TWO NEW TREMATODES FROM THE LOGGERHEAD TURTLE (*CARETTA CARETTA*)[1]

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Of twenty-one species of trematodes reported from the loggerhead turtle (*Caretta caretta* (Linn.)), seven species, *Cymatocarpus undulatus* Looss, *Orchidasma amphiorchis* (Braun), *Plesiochorus cymbiformis* (Rudolphi), *Rhytidodes secundus* Pratt, *Cricocephalus delitescens* Looss, *Wilderia elliptica* Pratt, *Pachypsolus tertius* Pratt, *Lophotaspis vallei* (Stossich), have been reported from America by Linton (1910), Pratt (1914), and Manter (1932). *Wilderia elliptica* Pratt is considered a synonym of *Diaschistorchis pandus* (Braun) by Mehra (1932).

The trematodes in the present collection were collected at the Biological Laboratory of the Carnegie Institution at Tortugas, Florida, in 1930 and 1932 by Dr. H. W. Manter under whose direction this study was made. Of the nine species (Table 1) studied, two species, *Styphlodora solitaria* (Looss, 1899) and *Pleurogonius trigonocephalus* (Rudolphi, 1809) have been previously reported only from marine turtles of the old world, and two species seem to be new.

TABLE 1.—*The species collected and their occurrence in the three turtles examined are shown in the following table*

Turtle	I	II	III
Cymatocarpus undulatus†	*		*
Hapalotrema synorchis n. sp.			*
Lophotaspis vallei		*	*
Orchidasma amphiorchis†	*	*	*
Plesiochorus cymbiformis	*	*	*
Pleurogonius trigonocephalus	*	*	
Pyelosomum longicaecum n. sp.			*
Rhytidodes secundus†			*
Styphlodora solitaria	*		

† Found also by Linton or Pratt.

Hapalotrema synorchis n. sp. (Fig. 1)

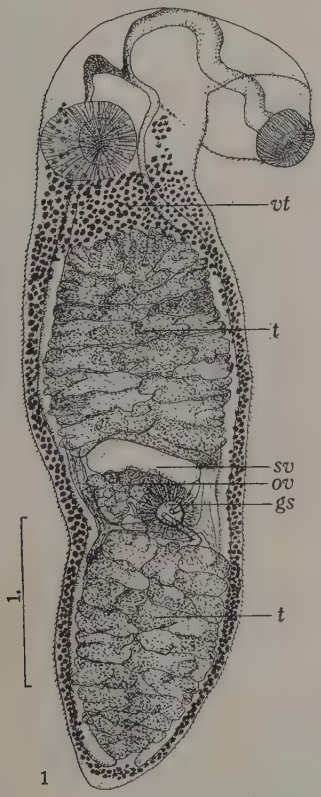
Diagnosis of *Hapalotrema synorchis*:

Size 6 by 1.5 mm. Body covered with spines. Oral sucker 383 μ in diameter. Ventral sucker 583 μ in diameter and located one-third body length from anterior end. No pharynx; esophagus a long, wide tube, narrowing toward bifurcation of ceca. Ceca extend to posterior end of body. Testes numerous, in two large, compactly massed groups, one anterior, the other posterior to the ovary. Seminal vesicle well developed, horizontally placed, immediately anterior to ovary, and not inclosed in cirrus sac. Cirrus present. Genital pore surrounded by well developed genital sucker to the left of, and ventral to the ovary. Deeply lobed ovary lies almost median between two groups of testes, a little more than two-thirds body length from anterior end. Seminal receptacle small, posterior to ovary. Laurer's canal present. Uterus short, opens at genital sucker. Vitelline ducts

[1] Studies from the Zoological Laboratory, the University of Nebraska, No. 182.

large. Vitelline follicles extending from anterior border of ventral sucker to posterior end of body, confluent posteriorly and anteriorly. No egg in uterus. Parasitic in the heart of *Caretta caretta* at Tortugas, Florida.

The genus *Hapalotrema* Looss, 1899, contains a single other species, *Hapalotrema constrictum* (Leared, 1862) from the heart of *Thalassochelys corticata* (= *C. caretta*).



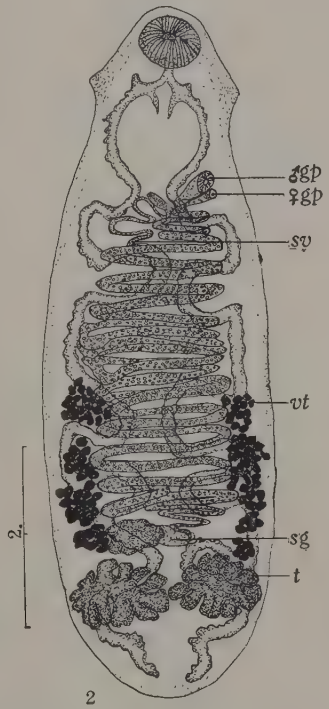


Fig. 1. *Hapalotrema synorchis* n. sp. Ventral view.
Fig. 2. *Pyelosomum longicaecum* n. sp. Ventral view.

The figures were drawn with aid of a micro-projector. The value of the projected scale is indicated in millimeters beside each figure. The following abbreviations are used: *gp*—genital pore. *gs*—genital sucker. *ov*—ovary. *sg*—shell gland. *sv*—seminal vesicle. *t*—testis. *vt*—vitellaria.

H. synorchis differs from *H. constrictum* in its large genital sucker, numerous testes massed together and in the more anterior extent and different distribution of the vitellaria.

A single specimen of *H. synorchis* was collected but its evident characteristics clearly distinguish it as new.

Pyelosomum longicaecum n. sp. (Fig. 2)

Diagnosis of *Pyelosomum longicaecum*:

Size 7 by 2.5 mm. Surface smooth. Anterior region of body provided with cephalic collar, the subventral lobes of which are connected by a dorsal ridge. Oral sucker 490 by 572 μ. Ceca extend in 6 serpentine undulations to posterior end of body. Undulations symmetrically arranged, coming almost together medianly to form 5 median loops and 6 outer loops (Fig. 2). Tendency toward short lateral branches on both sides of ceca. Male and female pores open close together to the left of the left cecum about one-third from anterior end of body. Cirrus sac present. Testes opposite, deeply lobed, posterior to ovary in posterior ¼ of body. Testes not entirely extracecal, but overlap the ceca ventrally. Ovary immediately anterior to right testis. Shell gland to left of ovary. Uterus passes forward in transverse folds covering inner foldings of ceca ventrally. Large vitelline follicles extend forward from testes for about ¼ body length, and are arranged in 4 groups on one side and 5 indistinct groups on the other. They cover outer foldings of ceca. Eggs measure 32–33 by 16–17 μ. Parasitic in the intestine of *Caretta caretta*.

The only other species in the genus *Pyelosomum* is *P. cochlear* Looss, 1899. *P. longicaecum* differs from *P. cochlear* in that the testes overlap the ceca instead of being entirely extracecal, the ceca are longer with 6 instead of 4 folds, the vitellaria extend only 1/4 body length instead of 1/3 to 1/2 body length, the ovary is to the right rather than median, and the eggs are only about 1/2 the size of the eggs of *P. cochlear*.

Type specimens of these two new species are deposited in the United States National Museum.

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ON THE RESISTANCE OF THE CAT TO SUPERIMPOSED INFECTION WITH THE ASCARID, *TOXOCARA CATI*[1]

MERRITT P. SARLES AND NORMAN R. STOLL

While little experimental work appears to have been done with the common ascarids of the cat and dog, knowledge gained about them should be of distinct value in advancing our understanding of such ascaris infections as of man and pig which yield less readily to experimentation. The present study was undertaken not only to obtain general information about ascaris infections in the cat, *Felis domesticus,* but to determine whether or not cats possess or can develop a resistance to infection with their common roundworm, *Toxocara cati.* The net result, that the cat has been found amazingly refractory to superimposed infection with this nematode, will be of interest to students both of ascaris infection and of host immunities to helminths. This finding is in the light of the recognition of the lag period which must elapse before an embryonated ascaris egg becomes infective, recently emphasized by one of us (1). Constant recourse has been made to lung migration tests for determining infectivity of eggs fed to the cats, and where possible results have been put on a quantitative basis. An abstract of a preliminary report of this work has already been published (2).

The animals used in the experiments were representative of domestic cats of this vicinity. Most of them, raised in the laboratory, were closely related. All harbored ascarids, but except for a few cases of hookworm and tapeworm in those not raised in captivity, the cats were free from other helminths. The occasional small coccidial infections present soon disappeared, as did the hookworm infections, when the animals came under laboratory control. The group as a whole was singularly free from intercurrent disease and no fatalities occurred during the experiments.

Experimental cats were kept in separate metal cages upon wire screens supported upon deep metal pans; pans and screens were scrubbed daily and each week completely sterilized. Added to these precautions, which alone seemed adequate to prevent reinfection, it was found that during the winter months when the experiments were run *Toxocara cati* eggs in feces dried and failed to develop when left exposed in the animal room. They required nearly three weeks to reach the infective stage even when in water on the laboratory table.

The cats were fed warm milk (made from milk powder) and com-

[1] From the Department of Animal and Plant Pathology of the Rockefeller Institute for Medical Research, Princeton, N. J.

mercial cat food daily with raw meat twice a week. The cat food contained meat and wheat flour as basic ingredients and was certified by the manufacturers to be free from anthelmintics, a claim which our observations, if anything, sustained.

Total feces passed on two consecutive days was collected each week from each cat. After mixing specimens thoroughly, fair samples were tubed in duplicate, 1 gm. of feces in 30 cc. of diluent. Flotation counts were relied upon with less than 200 eggs per gram of feces, and dilution counts in N/10 NaOH for those higher. The egg counting steps outlined by Stoll (3) were followed closely except that as a rule not more than one flotation cover or one dilution slide was counted.

These two day fecal specimens were also examined carefully for worms, and cages searched daily for ascarids which might have been vomited or passed. These when found were classified as large, medium, or small, and if microscopic were measured by ocular micrometer. Later, upon autopsy of an animal, the stomach and small intestine were removed, and contents and mucosal strippings screened through sieves of 20 and 40 meshes to the inch for macroscopic worms. Washings from this process were isolated in warm water in a Baermann funnel to recover microscopic ascarids and undeveloped larvae.

In cats and other animals fed *Toxocara cati* eggs the following criteria were used as proof of ascaris migration, (b) and (c) being considered crucial: (a) presence in lungs of characteristic ascaris hemorrhages; (b) finding of motile microscopic ascaris larvae in teased lung lesions; (c) recovery of active ascaris larvae by isolation of the finely minced lung in the Baermann apparatus; and (d) presence of characteristic ascaris hemorrrhages is sectioned and stained lung tissue.

The common ascarid of the cat, *Toxocara cati* Schrank, 1788, is the only ascarid we have found in cats of this region. This helminth commonly reached a length of 5 cm. for males and 8 cm. for females in our material. It is easily identified as an arrow-head ascarid by prominent cervical wings or alae which are short and abruptly rounded posteriorly. The esophagus has a very definite posterior muscular ventriculus, best seen in young or cleared specimens. The male tail is probular and the winged spicules may be as long as 2 mm. in large worms. The vulva is in the anterior fourth of the female body. Irregularly oval eggs have a fine mosaic shell marking. A series of 47 living eggs ranged 67 to 83 μ in long diameter with a mean of 73 ± 0.3 μ, and in short diameter from 53 to 73 μ with a mean of 62 ± 0.4 μ. Respective coefficients of variation were 3.8 ± 0.3 and 6.4 ± 0.5 per cent.

Most of our work was done with a single strain of *Toxocara cati*. This originated probably in the one or two cats from which all of our laboratory bred cats ultimately derived their infections while in a com-

mon breeding pen which had a dirt runway. Eggs from other strains have been used in a number of instances, but inasmuch as we have been unable to detect any difference in morphology or infectivity of the different strains they will not be discussed separately. Different methods of obtaining and culturing eggs were likewise without apparent effect.

For infection the embryonated eggs were washed several times in water and concentrated to that amount of suspension which would give approximately the desired dose in one or two 0.15 cc. samples, as shown by direct or subdilution count. No difficulty was experienced in getting animals to swallow these small amounts of fluid.

EXPERIMENTS AND RESULTS

I. Test of Infectivity of Eggs

Tovocara cati eggs were found to develop to infectivity on the laboratory table in water or one per cent formalin in 3 to 4 weeks, depending upon temperature. The lag period characteristic of *A. lumbricoides* (1) was also observed in this form between the first appearance of motile embryos in the eggs and actual infectivity as measured by lung migration in guinea pigs. For example: In a typical one per cent formalin culture eggs first contained moving embryos on the 13th day, and a definite sheath was first seen on larvae popped out of the eggs by pressure on the 17th day, but lung migration did not occur in guinea pigs fed eggs from this culture on days 14, 16, and 18, although it did follow the feeding of eggs on days 20, 22, 23, 24, 25, 27, 29, 30, and 100.

This migration, with resultant damage to the guinea pig lung, is acceptable as an adequate test of infectivity of eggs, since cats fed from the same lots of eggs and autopsied at the appropriate interval were comparable in regard to lung lesions. Infective eggs fed to mice, rats, and rabbits gave similar results, with large doses resulting in death from ascaris pneumonia.

Hatching of infective *Toxocara cati* eggs occurs soon after ingestion by cats. This was shown by recovery of empty egg shells and free larvae in contents of stomach and first foot of small intestine of each of 3 kittens autopsied, respectively, at one, two, and three hours after being fed 3000 eggs. In several other instances numerous free ascaris larvae were isolated in material vomited within a few hours after infection. However, a very considerable percentage of the eggs failed to hatch when fed to any of the test hosts mentioned, and could be demonstrated passed in the feces.

II. Attempts to Infect Kittens with Small Numbers of Infective Eggs, as Well as With Larvae in Guinea Pig Lungs

A cat with litter of 6 was secured the day after parturition and placed under conditions precluding accidental infection. Recovery of 2 *Toxo-*

cara cati 2.5 and 3.3 mm. long from the intestine of one kitten which died at 3 weeks of age, and of 2 additional specimens 0.9 and 3.0 mm. long from a second kitten which died at 4 2/7 weeks of age indicated that the litter had evidently become infected prenatally. Egg count and infection record for the other 4 animals is shown in Table 1. The dose

TABLE 1.—*Infection record and egg counts (eggs per gram in thousands) of kittens fed small numbers of Toxocara cati eggs, and larvae in guinea pig lungs*

Age of cats (weeks)	Cat A	Cat B	Cat C	Cat D
4	Fed 60 eggs			
6		0		0
8	0	0	0	0
9	0	0	0	0
10	0	0	0	0
10 4/7		Fed 550 eggs		
11	0	0	0	0
12	0	0	0	0.7
13	0	0	0.1	6.0
14	0	0	4.3	2.6
15	0	0	5.0	5.0
16	0	0	5.1	3.2
17	0	0	4.7	3.2
18	0	0	8.9	1.5
20	0	0	9.4	3.3
20 4/7	Fed 500 eggs		Fed 500 eggs	
21	0	0	4.9	1.9
21 4/7		Fed 25–50 larvae in guinea pig lung		Fed 25–50 larvae in guinea pig lung
22	0	0	3.2	0
23	0	0	2.2	0
24	0	0	2.1	0
25	0	0	0	0
25 3/7	Autopsied. 1 large ♂ recovered	Autopsied. No worms	Autopsied. 1 large ♂ recovered	Autopsied. No worms

of 60 eggs fed Cat A was from a 35 day culture and was not tested for infectivity since comparable cultures had been infective at that age. The eggs fed Cat B were from a 30 day culture proved infective by guinea pig test. If these two doses of eggs had any effect at all it was inhibitory because ascaris eggs never appeared in the feces of these cats, whereas they were found in the feces of Cats C and D at respectively 13 and 12 weeks of age. A second unsuccessful attempt to infect Cat A was made at 21 weeks of age when with Cat C it received 500 eggs from a 100 day old culture. A guinea pig fed 1000 eggs from the same culture had numerous ascaris hemorrhages in its lungs when killed 6 days later, and portions of these lungs were fed to Cats B and D. Finally at 25 weeks of age, when the two cats which earlier had spontaneously developed egg counts now ceased to pass eggs, all 4 were killed. The single large male ascarid recovered from Cat C was too large to have resulted from the dose of eggs fed to this cat, and like the similar ascarid recovered from Cat A it probably represented a remnant of an infection acquired prenatally. If, as seems highly probable, the eggs passed by Cats C and D

represented the maturing of a prenatal infection, 12 to 13 weeks was required for development of *Toxocara cati* to the egg-laying stage. In the absence of definitive data, this indirect evidence of a long developmental period is of special interest because it was, (1) determined on very young and therefore supposedly highly susceptible animals, (2) supported by later experiments, and (3) obtained on infections consisting of both male and female worms (the eggs passed were fertilized). Duration of infection in these same 2 kittens was respectively 12 and 10 weeks.

III. Graded Doses Fed to Kittens

Small single doses having failed to infect, single doses of 300, 3000, and 30,000 eggs were fed to 3 of 4 kittens of unknown parasitic history but judged by weight (0.9–1.0 kilo) to be about 5 months old. The eggs consisted of equal amounts of 3 tap water cultures 66, 75 and 153 days old; and had been proven infective by separate guinea pig migration test. At autopsy 6 weeks later 20 large ascaris but no small ones were recovered from the small intestine of the control which had passed ascaris eggs throughout the experiment. The kittens fed 300 and 3000 eggs likewise passed ascaris eggs throughout the experiment and at autopsy harbored, respectively, 1 and 8 large worms, but also 2 and 3 very small ascarids 1–3 cm. long. The fourth kitten, fed 30,000 eggs, passed no ascaris eggs and at autopsy its intestine contained only 3 very small ascarids 1 cm. long. Since the worms recovered from the kittens of this experiment fall solely into two extreme sizes, and the uninfected control alone failed to exhibit signs of new infection, the conclusion seems warranted that each of the doses fed initiated development of a new infection of very small size.

IV. Attempt to Infect Cats of Three Age Groups by Single and Repeated Doses of Eggs

Small single doses and graded single doses of eggs having failed to produce infections of substantial size in kittens, this experiment was set up to test the ability of repeated doses of constant and increasing size and also single doses of graded sizes to establish new ascaris infection in cats of three different age groups. Twenty-one cats were divided into 4 groups and infected as shown in Figs. 1–3.

The eggs used came from cultures 27–48 days old, and each batch of eggs produced lung lesions with demonstrable ascaris larvae in guinea pigs autopsied 5–6 rays after infection. Infectivity of the final Test Dose eggs was established by a graded series of doses, and resulting graded severity of lung lesions in, as well as recovery of increasing numbers of larvae from the lungs of 3 guinea pigs fed 5000, 10,000, and

15,000 of the same eggs. The guinea pig which received the largest dose, approximate in size to the Test Dose given the cats, died 2 days after infection; 1500 ascaris larvae were isolated from its hemorrhagic lung and 5300 from liver. Cats 14, 17, and 20, autopsied 5 days after receiving the Test Dose, also exhibited fresh ascaris lung hemorrhages from which larvae were recovered.

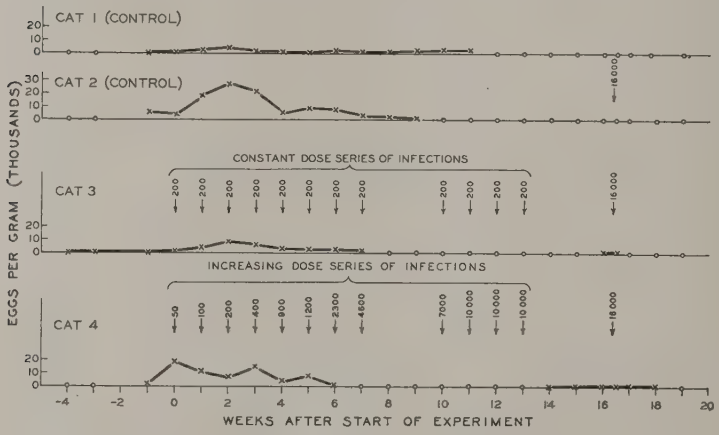

Figure 1. Egg count and infection record for a litter of four kittens (see text).

Figure 1 shows the infection and egg count record for *4 kittens* which were obtained one month before the experiment was started, when, judging by weight, they were approximately four months old. All 4 harbored natural infections. These persisted 9 and 11 weeks in the two control kittens but only 6 and 7 weeks in the two fed weekly doses of eggs. Following the loss of the natural infections no ascaris eggs were found in feces of the controls. Both experimentally infected kittens passed small numbers of eggs during later weeks of the experiment but these ceased after administration of the Test Dose. As measured by egg count, weekly doses of constant and increasing size produced in these young kittens definite ascaris infections of smaller size and shorter duration than natural infection, and appeared to shorten the duration of natural infections.

Figure 2 summarizes graphically, at half the scale of Figure 1, the egg count and infection record of *9 young cats*. These animals had been raised in captivity by the adult cats of the next group and like them had been inadvertently exposed to natural reinfection in the breeding unit. At start of the experiment they were approximately eight months

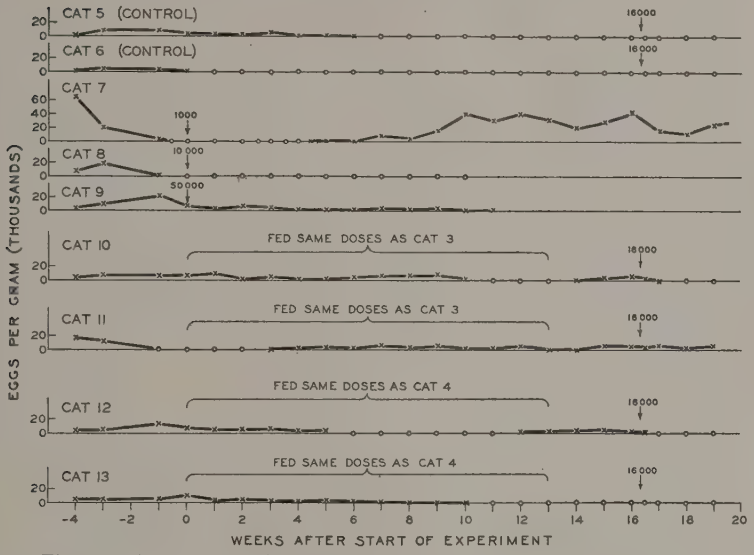

Figure 2. Egg count and infection record for nine young cats (see text).

old, and during the preceding 4 months had been kept under conditions preventing further infection. Natural infections harbored were apparently in different stages of development since some disappeared soon while others persisted for many weeks. The two controls showed no recurrence of infection. There was great variation in the duration of the natural infections of the other cats, and in four of them loss of the natural infection was followed after an interval by a secondary series of egg counts. In the case of No. 7 and No. 11 it is doubtful if these eggs could have been due to worms developing from the experimental infections which the cats had received since in the preliminary experiment cited in Section II the first eggs did not appear in kittens until twelve or thirteen weeks even after presumptively prenatal infection. With Cats 10 and 12, however, a sufficient interval had elapsed so that the secondary series of egg counts may have been due to the experimental infections. It also seems probable that the final undepicted increase in egg count of No. 7 (reaching 33, 9, 10, 28, and 45 thousand eggs per gram on the weeks 20, 21, 22, 23, and 24, respectively), resulted from experimental infection. The Test Dose of eggs given to 6 cats gave rise to no increase in egg count. On the contrary it was followed by decreases in the existent count in two cases. As measured by egg counts, attempts to infect young cats by single and repeated doses of eggs appeared to have established some new infection in 3 of 7 cats.

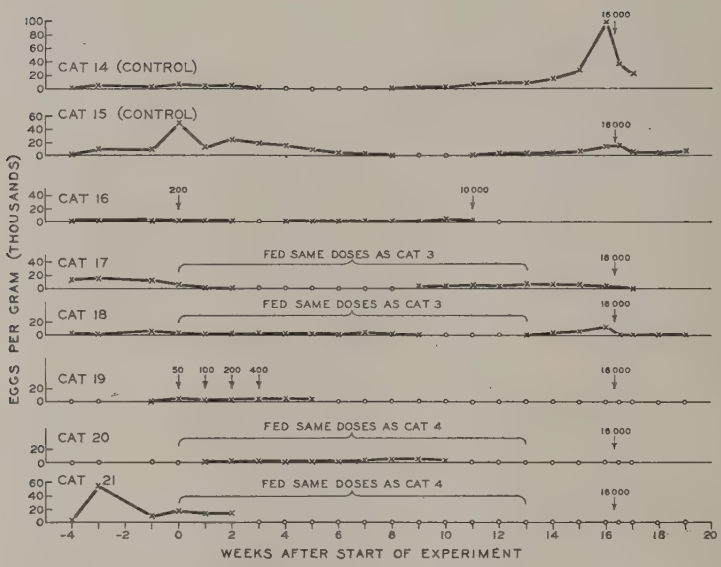

Figure 3. Egg count and infection record for eight adult cats (see text).

Figure 3 shows the doses of eggs fed to *8 adult cats* and the numbers of ascaris eggs found in the feces of these cats at weekly intervals. Six of these cats were approximately 1½ years old at the time of experiment, but Nos. 17 and 15 were at least twice as old. All had been removed from the common breeding unit, where they had been unintentionally exposed to natural reinfection with cat ascaris, and kept on screens for one month preceding the experiment. All but one showed the presence of natural ascaris infection during this preliminary period. In the controls infection was gradually lost. Then followed appearance of a secondary series of egg counts which we interpret as evidence of delayed development of worms acquired naturally, since chance of accidental infection during the experimental period was believed to be entirely eliminated. Secondary egg counts did not occur in Cats 19, 20, and 21, fed increasing numbers of eggs. In Cats 17 and 18, fed weekly doses of constant size, the secondary egg counts, although present, were small. The effects in these two groups of animals then suggests the action of some mechanism by which repeated parasitic assault prevents development of worms already established. In the 4 cats (Nos. 14, 15, 17, and 18) passing ascaris eggs when fed the Test Dose, a decrease occurred in the egg count. By this measure the adult cats failed to develop new ascaris infections when fed weekly doses of small or increasing size, a

TABLE 2.—*Number and size of worms recovered from cats of three age groups when exposed to three different types of infection with Toxocara cati*

Period	Size of worms	I Control cats						II Single infections				III Constant doses					IV Increasing doses					
		1	2	5	6	14	15	7	8	9	16	3	10	11	17	18	4	12	13	19	20	21
Preliminary	Large	–	–	2	–	–	–	1	3	–	–	–	–	1	–	–	–	–	1	–	–	–
	Medium	–	–	1	–	–	–	–	–	–	–	–	–	–	2	–	–	–	–	–	–	–
	Small	–	–	–	–	–	–	–	–	–	–	–	–	–	–	1	–	–	–	–	–	–
Experimental	Large	3	5	1	1	7	5	2	2	5	7	5	5	4	14	6	1	2	1	1	1	4
	Medium	3	1	5	–	–	1	1	–	1	–	–	–	3	4	–	–	–	2	–	1	3
	Small	–	–	–	–	–	2	–	–	1	–	13	1	4	6	1	–	–	22	–	–	–
At autopsy	Large	–	–	–	–	–	3	3	–	3	–	–	–	2	1	1	–	–	–	–	–	–
	Medium	–	–	–	–	–	–	3	–	4	–	–	–	8	2	–	–	–	2	–	–	–
	Small	–	–	–	–	–	–	12	18	7	–	8	4	2	2	1	15	11	5	–	–	28
	Larvae from small intestine	–*	3D 2U	1U	–	2U	1U	–*	–*	–*	–	–	–	1D	6D 19U	1D	2U			Not exam.	3U	1D 1U
	Larvae from lung	–*	240	25	15	250	125	–*	Not exam.	Not exam.	8	80	1	2	2100	50	161	250	27	25	3	78

* = Received no test dose.
D = Developing larvae.
U = Undeveloped larvae.

single dose of 200 eggs, or four small increasing doses. On the contrary, weekly increasing doses seemed to prevent the development of worms, which, to judge by the controls, were already present.

A more direct measure of the extent to which experimental feedings of *Toxocara cati* eggs caused establishment of new infection is afforded by comparison of the number of worms recovered from the cats of the four infection groups during the experiment and at autopsy. This information is summarized in table 2.

At autopsy only one of the control cats, No. 15, harbored sizeable ascarids and the 3 worms in this cat were large. Obviously these had been present and producing eggs even before feeding of the Test Dose. Ascaris larvae of microscopic size were recovered in small numbers from the intestines of some cats of every age and infection group, and in larger numbers from the lungs of every cat which received the Test Dose, but the number was variable and gave no indication of any additional degree of immunity resulting from experimental infection. Some larvae recovered from the small intestine had grown perceptibly: the longest from control cats measured 1.2 mm. and from the other cats 1.5 mm. Larvae recovered from lungs had not increased in length perceptibly. This apparent failure of *Toxocara cati* to grow during migration is in marked contrast to the striking growth of larvae of *Ascaris lumbricoides* of swine during migration in many experimental animals. The following data summarize statistically a small number of measurements on living larvae of *Toxocara cati* in water and a more extensive series on larvae fixed in glycerin-alcohol. (Measurements of length in micra: R = range, M = mean, C. V. = per cent coefficient of variation.)

Measurements on Living Larvae of Toxocara cati:

For 16 larvae popped by gentle cover glass pressure out of infective eggs cultured for 22, 25, and 30 days: R = 335–420, M = 377 ±3, C. V. = 4.0 ± 0.5.

For 23 larvae recovered from 12 guinea pigs' lungs at 5–7 days after infection: R = 335–455, M = 402 ± 5, C. V. = 8.3 ± 0.8.

For 11 larvae recovered from mouse lungs and liver, and rabbit lungs at 1–5 days after infection: R = 375–470, M = 410 ± 5, C. V. = 6.1 ± 0.9.

For 7 larvae recovered from the lungs or liver of 4 cats at 5–8 days after infection: R = 390–460, M = 430 ± 5, C. V. = 4.0 ± 0.8.

Measurements of Toxocara cati Larvae Fixed in Glycerin-Alcohol:

For 75 larvae popped out of infective eggs cultured 25 and 35 days: R = 230–390, M = 323 ± 3, C. V. = 11.0 ± 0.6.

For 130 larvae recovered from 10 guinea pigs' lungs at 2–7 days after infection: R = 330–410, M = 365 ± 1.1, C. V. = 4.9 ± 0.2.

For 170 larvae recovered from 5 cats' lungs at 5, 6, and 21 days after infection: R = 350–440, M = 389 ± 0.8, C. V. = 4.0 ± 0.2.

The mean length of the larvae recovered from animals was significantly greater (four or more times probable error of difference) than for larvae from infective eggs, for measurements of both living and fixed larvae. The increase is relatively small, amounting to 7, 9, and 14 per cent, respectively, for living larvae isolated from guinea pigs, mouse and rabbit, and cats, and to 13 and 20 per cent for fixed larvae recovered from guinea pigs and cats. This amount of change was not perceptible to the eye and is insignificant when compared to the tremendous growth (several hundred per cent in length) manifested by larvae of the pig ascarid under the same conditions. The mean length of small numbers of larvae recovered from (a) guinea pigs, (b) mice and rabbits, and (c) cats, did not differ significantly from each other when the larvae were measured without fixation. For fixed larvae the mean length of those from cat lungs was significantly larger than those from guinea pig lungs.

Number of macroscopic worms recovered from cats of different age groups: No worms were recovered from the *kittens* (Table 2, Nos. 1–4) during the preliminary period, but during the experiment some worms were recovered from all of them, and No. 3, fed weekly doses of small constant size, gave evidence of newly developing infection by passage of numerous small worms. At autopsy both controls were negative for ascarids, whereas numbers of small ones were recovered from both of the repeatedly infected animals. A few ascarids were recovered from the *young cats* (Table 2, Nos. 5–13) of all 4 infection groups in the preliminary period. During the experiment many more were recovered from all 9 cats, and Nos. 11 and 13, fed weekly doses of eggs, gave indication of some development of new infection by passing a significantly larger number of worms than the others. At autopsy the controls had no macroscopic worms but numbers were recovered from each of the experimentally infected cats; most of these ascarids were of small size, even in Nos. 7, 8, and 9 which had been fed a single dose of eggs, respectively 24, 10, and 11 weeks previously. Experimental feeding of eggs also produced demonstrable parasitism in 3 of 6 *adult cats* (Table 2, Nos. 14–21) as evidenced by the presence of small worms in their intestines at autopsy, whereas of 2 controls one harbored only 3 large worms, the other none. The latter had recently vomited 4 large ascarids and passed another in the feces used for its final egg count, which showed many eggs.

Comparison by infection groups of numbers of worms recovered: A clearer conception of the extent to which parasitic infection was established by single and repeated doses of eggs is afforded by comparing the number of worms recovered from cats of each of the 4 infection groups

(table 2) as well as by contrasting the average number of worms recovered per cat in the same groups (shown graphically in figure 4). Worms

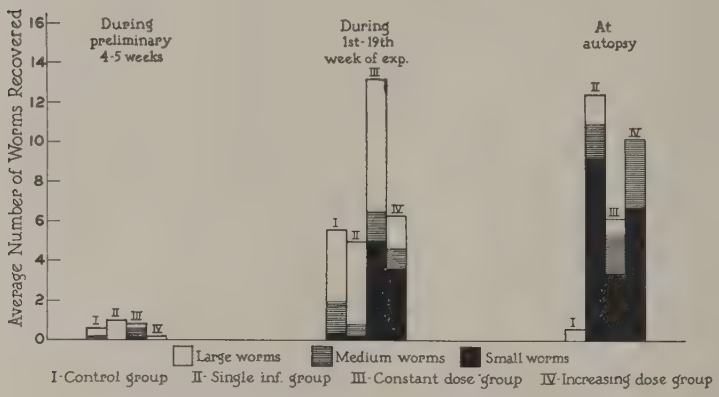

Figure 4. Average number, and size of worms recovered from each group of cats.

recovered *during the preliminary period* were predominantly of large and medium size and the average number per cat was approximately identical for all 4 infection groups. *During the experiment* worms were passed in feces and vomited at irregular intervals by cats of all infection groups, but in addition considerable numbers of worms were recovered from the experimentally infected animals following feeding of the first few doses of eggs, and after the Test Dose. Groups III and IV, which had received repeated doses of eggs, differed strikingly from Groups I and II in the large percentage of small worms recovered; more than twice as many ascarids were obtained per cat from Group III as from the other 3 groups. *At autopsy* a total of 3 large worms was present in one of 6 control cats, whereas 142 worms were obtained from 12 of the 15 experimentally infected cats, and most worms from Groups II and III and all from Group IV were of small and medium size.

Effect of ascaris infection on cats: The weights of the infected cats in each group did not differ significantly from those of the controls. As mentioned previously, vomiting occurred quite frequently in cats fed eggs, but also occurred occasionally in controls. Repeated spasms of coughing and sneezing were observed in all adult cats fed eggs but not in controls, and some coughing and sneezing occurred in 3 experimentally infected young cats. Although "fits" are often attributed to "worms" none were observed in these 21 cats, each of which harbored ascaris for a considerable period while under close observation.

DISCUSSION

Uniform failure of cats to become parasitized to any considerable degree when fed *Toxocara cati* eggs either in small or large single doses or in weekly doses of constant or increasing size, raises the question of the mechanism involved in this resistance. Its exact nature is not understood, but it is possible to narrow the problem considerably by evaluating positive and negative findings. Failure to infect can not be ascribed to chance use of a non-infective strain of parasite, nor to an abnormally refractory host strain, because several strains of host and parasite were used, without demonstrable distinction, and all gave identical results. Neither can it be attributed to failure to use truly infective eggs, since repeated tests on cats, guinea pigs, etc., showed normal hatching and migration of larvae through the lungs and back to the intestine. Postulation of age resistance or acquired immunity resulting from repeated infection seems unwarranted, because young kittens were found refractory as well as young and old cats, and some of the cats studied had almost certainly been exposed only to prenatal infection. Moreover, experimental test for age resistance or immunity acquired as the result of experimental infection failed to demonstrate either. Consequently, the possibilities narrow down to two main groups of factors: (a) those acting to limit the number of larvae reaching the intestine, and (b) those acting on the larvae after they have reached the intestine. Our results indicate that the first group prevent all but a few larvae from reaching the intestine: only part of the eggs fed to a cat liberate their larvae while passing through the alimentary tract, and some eggs and larvae are often vomited; since only a very small fraction of the larvae in the dose is ever recovered from the lungs and still less from the intestine, it seems probable that the larvae which do succeed in escaping from the eggs do not all penetrate the small intestine, and that of the few which do the majority are lost in host tissues during migration. We are ignorant of the nature of the second group of factors but the evidence is fairly convincing of their ability to delay or prevent completely the growth of larvae which have reached the small intestine and to cause the expulsion in vomitus or feces of ascarids in all stages of development. As these reactions occur in the absence as well as in the presence of worms from previous infection, they would appear to have their origin in the host and not be attributable solely to parasitic crowding.

Unlike experimental infections with dog hookworm, *Ancylostoma caninum* (4), the common sheep stomach worm, *Haemonchus contortus* (5), and *Trichostrongylus calcaratus* in the rabbit (6), in which percentage development is high and production of fatal infestations rela-

tively easy, the percentage of *Toxocara cati* eggs which developed in cats was very small, and clinical effect slight. It is possible that the cat is by nature only slightly susceptible to infection with *Toxocara cati*. However, final solution of the question as to whether the cat's resistance to ascaris infection is natural or acquired and whether age and repeated infection play a part in older animals must await further research on worm-free stock. The problem of obtaining such animals will be difficult, as our unsuccessful attempts have demonstrated, because of the probable occurrence of prenatal infection, the extremely slow developmental period during which fecal examination gives no hint of the presence of infection, the variable and disappointingly small infestations which result even from carefully controlled and repeated feedings of eggs, and the difficulty of demonstrating any increased immunity acquired as the result of age or experimental infection because of the high resistance already present in parasitized cats.

SUMMARY

Cats, of different age groups and carrying natural infestations of *Toxocara cati,* were found to possess a uniformly high degree of resistance against attempts to superimpose infection with this arrow-head ascarid. This conclusion comes from both egg and worm counts, after feeding eggs shown to be infective by guinea pig migration test, in single doses of small or large size, weekly doses of small and constant size, or increasing size, and larvae in migration stages in guinea pig lungs. Two groups of factors appear involved in this resistance: (1) Those limiting drastically the number of larvae reaching the small intestine and including failure of some eggs to hatch, loss of some eggs and larvae through vomiting and passage in feces, and loss of some larvae during migration through the host's tissues. (2) Those acting on ascarids which have migrated and returned to the small intestine, and including apparent prevention of growth, or retardation of growth with shortened duration of infection and decreased egg production of worms, and expulsion by vomiting or passage in feces of worms in all stages of development.

In cultures, developing *Toxocara cati* eggs containing motile embryos only became infective, as measured by ability to undergo lung migration in guinea pigs and cats, after a lag period approximately equal to the time necessary for embryonation itself under given conditions of culture.

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FURTHER OBSERVATIONS ON THE RELATION OF CERTAIN CARBOHYDRATES TO *TRYPANOSOMA EQUIPERDUM* METABOLISM[1]

HILDRUS A. POINDEXTER

In a previous report it was shown by Poindexter (1) that artificial hyperglycemia produced by intraperitoneal injections of glucose shortened the prepatent period and increased the rate of reproduction of *Trypanosoma equiperdum*. It was also observed that when two groups of guinea-pigs were infected with *T. equiperdum* organisms of equal number and virulence, the group that was on a lower carbohydrate or starvation régime lived on the average of from twenty to twenty-seven per cent longer after infection than the group on the normal diet. These *in vivo* observations seem to agree with the *in vitro* observations of Schern (2), and others, who believe that there is a significant correlation between the amount of readily absorbable carbohydrates and the rate of growth and activity of trypanosomes of the blood.

Believing that the fermentable carbohydrates of the blood formed an essential part in trypanosome metabolism, we decided to do a series of *in vivo* and *in vitro* experiments to further appraise the value of this belief. The *in vivo* experiments were done by repeated intraperitoneal injections of insulin,[2] which almost depleted the blood of the infected animals of fermentable carbohydrates. The injection of insulin into infected animals altered the trypanosome metabolism by decreasing the rate of reproduction of the trypanosomes, as shown both by the change in the numbers in the blood and by the coefficient of variation shown by the method used by Taliaferro and Taliaferro (3). There was also an increase in the survival time of the infected animals. The *in vivo* experiments were done by making a comparative record of the survival time, length of retention of virulence, and rate of motion of the trypanosomes after removal of small amounts of infected blood from the animals and diluting them with various isotonic solutions containing different amounts of fermentable carbohydrates.

PRELIMINARY TEST

Thirty-nine guinea-pigs were used for the *in vivo* experiments. Blood sugar, according to Folin and Svedberg's 1930 Ferricyanide Micro Method (4), was done on each guinea-pig prior to infection. This figure served as normal blood sugar for each animal and this average served

[1] From the Department of Bacteriology, Preventive Medicine and Public Health of Howard University School of Medicine, Washington, D. C. I wish to express my appreciation to Doctors Paul B. Cornely, of Howard University, for

as the group average for the normals. The thirty-nine guinea-pigs were then divided into four groups. Groups one, two, and three each contained ten guinea-pigs, which were respectively injected intraperitoneally with 25,000, 125,000, and 250,000 fully virulent *T. equiperdum* organisms. Group four, which contained nine guinea-pigs, was not infected and served as non-infected controls. This fourth group was appropriately marked and submitted to the same environmental conditions as the other groups. In order to determine the amount of insulin necessary to cause non-fatal and fatal insulin shock, in guinea-pigs, we used twelve animals from a group similar to the thirty-nine noted above. It was found that the non-fatal shock dose was one unit for seventy-one grams of body weight, while the fatal dose was one unit for sixty grams of body weight. It was observed that the doses did not vary materially between the non-infected and the infected animals, except that in the former, the return of the blood sugar to normal after the non-fatal shock was more rapid than with the latter. In the infected animals, the amount necessary for shock decreases as the infection progressed. This decrease in the amount of insulin required to produce shock correlates highly with the decrease in the blood sugar.

EXPERIMENTAL

In vivo: The guinea-pigs of groups one, two, and three were each divided into sub-groups of five each. Twice per week each of the animals of the sub-group was given one unit of insulin per seventy-five grams of body weight. Blood sugar and the number and percentage change of trypanosomes were determined on each animal just before and one to two hours after insulin injections. The average results of group one are shown in Table 1. All blood sugar determinations were done on blood taken by cardiac puncture. The animals of the other sub-groups did not receive insulin. Similar determinations were done on them at weekly intervals, and the results are given in Table 1 for comparison.

The survival time of the infected animals in group one of the sub-groups without insulin were as follows: one died during the eighth week, two during the twelfth week, and two during the thirteenth week of the infection. For the insulin sub-group, the deaths were as follows: one during the thirteenth week, two during the sixteenth week, and two during the seventeenth week. The usual injection of one unit per seventy grams of body weight did not cause shock during the early part of the infection, but by the fourth week of the infection one of the guinea-

certain mathematical criticism of the data obtained from the measurements, and Lloyd Newman and Mr. Rutherford B. Stevens, of Howard University, for checking some of the chemical results.

[2] The insulin (Iletin, U-20) was supplied through the courtesy of the Eli Lilly Research Laboratory, Indianapolis, Indiana.

TABLE 1.—*The effect of insulin injections on the changes in blood sugar and trypanosome count in guinea-pigs infected by intraperitoneal injections of 25,000* T. equiperdum.

Weeks of infection	Mgms. of blood sugar			Average number of trypanosomes per cmm.		Average % change over previous week	
	Without insulin injection	With insulin injection		Without insulin injection	With insulin injection	Without insulin injection	With insulin injection
		Before injection	After injection				
Before injection	87.1	88					
1	88	M 83	54				
		F 86	51	0	0		
2	93.4	M 87	50				
		F 85	50	27	6		
3	81	M 80	41				
		F 83	47	11,200	3,800	41381	63233
4	75	M 71	42				
		F 70	40	23,600	10,100	111	166
5	75.8	M 66	43				
		F 66	41	27,000	17,000	16	68
6	70.9	M 64	47				
		F 63	47	31,400	17,900	16	5
7	64	M 60	45				
		F 60	41	15,100	18,000	−52	.5
8	62	M 60	46				
		F 64	45	2,900	19,300	−81	7
9	62.1	M 60	40				
		F 60	43	3,600	24,000	24	24
10	51	M 56	38				
		F 60	41	28,000	9,600	678	−60
11	54	M 55	34				
		F 59	41	39,100	9,000	40	−6
12	56	M 59	42	37,000	7,800	−5	13
13	57	M 53	41	26,600	11,000	−28	41
14		M 53	40		21,000		90
15		M 55			23,800		13
16		M 57			21,000		−12
17		M 39			37,000		76

M = Monday; F = Friday.

pigs went into non-fatal shock, and by the sixth week, all of this subgroup were non-fatally shocked by this dose.

This dose was then reduced to one unit of insulin per ninety grams of body weight. During the eleventh week of the infection, one of the animals went into non-fatal shock with this dose. The unit was not reduced further, but the frequency of the injections was reduced to once per week during the remaining course of the infection.

The coefficient of variation test was done by making camera lucida tracings of 100 trypanosomes weekly from each infected guinea-pig in group one. The microscope was standardized with the camera lucida mirror, a Leitz 1/12a oil immersion objective and 8 x B ocular into which was placed a finely ruled micrometer. This combination gave good resolution and camera lucida tracings of trypanosomes were made. The tracings were measured in m.m. and with the aid of the micrometer the objects were expressed in microns. The transferences were made from a table that showed the number of equivalent microns of the objects

on the slide which correspond to the number of m.m. of the camera lucida tracing on the paper.

The slides were permitted to dry in air, were then fixed with methyl alcohol for fifteen minutes and stained for thirty minutes with Giemsa's blood stain. We were able to see the parts of the trypanosome clearly even to the end of the flagella. The nucleus and parabasal bodies frequently showed various stages of division. The blepharoplast in some cases was not so clear. It was at times included in the parabasal body staining area or could only be located by the abrupt ending of the undulating membrane at its point of origin. It was shown by Taliaferro in 1922 that while osmic acid vapor for fixation of the slide before staining prevented some of the shrinkage in size of the organisms the uniformity of the shrinkage in slides not so fixed made other methods satisfactory for coefficient of variation determination. On that basis we used the technique noted above.

Rapidly dividing trypanosomes show both a variation in length and width. In an effort to show the rate of multiplication as an expression of these two variables, 100 trypanosomes were taken from each of two different guinea-pigs for two successive weeks. The mean variations of these 400 organisms were expressed as the average variation in the sum of the products of the following measurements: posterior end to parabasal body; parabasal body to nucleus; nucleus to anterior end and anterior end to end of flagellum, multiplied by the respective widths in each of these distances. Coefficient of correlation was done on these 400 measured trypanosomes according to the product of the length times the width, on the length alone and the width alone, compared to the rate of multiplication as shown by the changes in the number from week to week. It was found that if the flagella was not included in the measurement the variation of length time width (*lw*) gave a higher positive correlation but when the flagella was included due to small width, it was found best to use the variation in the lengths alone as an expression of the changes in the rate of multiplication. The personal error was also less with the length measurements. The standard deviation when length alone was used was determined by the formula: $\sigma = \sqrt{\frac{\sum x^2}{n} - M^2}$, instead of $\sigma = \sqrt{\frac{\sum (lw)^2}{n} - M^2}$, where in the former x = length of trypanosomes and M = mean length, in the latter l = length; w = width of the trypanosomes measured and M = the mean of lw. n = the number of trypanosomes measured in both formulae.

Table 2 shows the findings from ten weekly examinations beginning the second week after infection and continuing for the next ten weeks. There were 9,700 trypanosomes measured; one animal died during the

TABLE 2.—*The effect of insulin injection on the average means, standard deviations and coefficient of variation for total length and the number of trypanosomes per cm. of blood determined at weekly intervals for the guinea-pigs in group one.*

Weeks after injection	With insulin injection				Without insulin injection			
	Number of trypano-somes per cmm. of blood	Mean length	σ	C.V.	Number of trypano-somes per cmm. of blood	Mean length	σ	C.V.
1	0				0			
2	6	24.301	2.031	8.357 ± .289	27	24.320	2.551	10.489 ± .315
3	3,800	24.416	2.156	8.830 ± .295	11,200	24.613	2.960	12.026 ± .341
4	10,100	24.526	2.436	9.969	23,600	25.010	3.023	12.087
5	17,000	24.798	2.601	8.270	27,000	25.200	2.911	11.908
6	17,900	25.000	2.211	8.844	31,400	26.415	2.431	9.203
7	18,000	24.830	2.037	8.163	15,100	25.901	2.431	9.385
8	19,300	25.300	2.375	9.391	2,900	26.512	2.501	9.056
9	24,000	25.911	2.752	10.620	3,600	26.321	2.571	9.767
10	9,600	25.387	2.231	8.787	28,000	25.237	3.317	13.144
11	9,000	24.951	2.179	8.733 ± .289	39,100	26.091	3.000	11.536 ± .351

eighth week which prevented the measurement of 10,000 as we set out to do.

The difference in the coefficient of variation amounts to a sufficient number of multiples of the probable errors to make the difference definitely significant.

The method of measuring the rate of division as shown by Robertson (7) was not sufficiently studied in our work to permit a significant comparison.

When equal numbers of trypanosomes were transferred without delay from infected guinea-pigs to susceptible rats, the course of the infection on the average was more rapid in the rats receiving the trypanosomes from the non-insulin sub-groups. This shows a slight decrease in virulence of the trypanosomes of the insulin group. This decrease in virulence (length of time necessary to cause death in rats) shows a high correlation with the changes in the rates of multiplication of the trypanosomes, both in the guinea-pigs from which the organisms were obtained, and the rats into which they were inoculated.

The course of the infection in each of the sub-groups of guinea-pigs showed relapses. The number of trypanosomes present in the peripheral circulation at each peak was, however, greater in the non-insulin than in the insulin sub-group. The differences in the rate of multiplication of the organisms were determined both by the percentage changes, as shown by the numbers of organisms in the circulation at regular intervals during the course of the infection, and by the coefficient of variation at each of these intervals.

The thermoprecipitation test on the spleen or liver extracts showed a more pronounced reaction on the non-insulin sub-groups. The spleens

in this sub-group were larger and more firm. This sub-group was interpreted as showing a more active defense than the insulin sub-groups.

The chief differences in the results of the animals in groups two and three from those of group one, described above, are those due to the larger initial injections. The infection ran a more rapid and earlier fatal course with a shorter prepatent period, more rapid decrease in the blood sugar as the infection progressed, larger numbers of trypanosomes in the blood at various peaks, and less tolerance for fatal insulin shock occurring with a dose that would only cause mild shock in animals in similar periods of the infection in group one. The return of the blood sugar to its preinjection level was neither as rapid nor as complete in groups two and three as in group one. The animals in group four were used chiefly to check the results of observations in group one. They were later used for reservoir for some of the *in vitro* experiments.

In vitro: In order to determine the effects of glucose solution controlled by certain other isotonic solutions on certain metabolic factors *in vitro,* seven solutions were used: (a) Physiological saline; (b) Glucose, 0.25 per cent solution; (c) Locke's solution; (d) An isotonic solution containing all of the inorganic elements of Locke's solution except the glucose; (e) Locke's solution to which has been added 1 per cent of sterile normal rat's serum; (f) Locke's solution to which has been added 1 per cent of sterile immune rabbit's serum; and (g) Locke's solution with normal rabbit serum. 1 per cent suspensions were made of the blood and trypanosomes drawn from infected animals in each of the

TABLE 3.—*Table showing the effect of temperature, oxygen and certain other elements on the survival time and rate of motion of* Trypanosoma equiperdum *in vitro.*

Solution	At room temperature 22–25° C.				In the incubator at 37.5° C.			
	Under aerobic conditions		Under anaerobic conditions		Under aerobic conditions		Under anaerobic conditions	
	Average survival time in hours	Average rate of motion in mm. per minute	Average survival time in hours	Average rate of motion in mm. per minute	Average survival time in hours	Average rate of motion in mm. per minute	Average survival time in hours	Average rate of motion in mm. per minute
A ...	6	1.6	8.5	1.6	6.75	1.3	9	1.5
B ...	19	2.1	22	2.4	10	1.4	20	2.1
C ...	21	3.7	33	3.1	18	4	26	3.2
D ...	7	2.3	8	2.3	7.3	2.4	7.5	2.1
E ...	21	3.1	37	2.9	14	1.8	17	1.5
F ...	3	0	3.5	0	2.4	0	3.6	2.0
G ...	17	2.6	26	2.8	15	1.9	19	2.0

Many of the vials containing the trypanosome suspensions were also contaminated with the common saprophytic bacteria. This occurred during the drawing of blood from the heart, tail, or ears. This contamination is believed to have influenced the results of both the survival time and rate of motion. In general the trypanosomes from the peripheral circulation were more motile and survived longer.

above solutions. Table 3 shows the survival time as determined by repeated microscopic examinations of drops of suspension removed from vials containing them. The recorded survival time represents the last observation when motile trypanosomes were observed in the suspension. This table also shows the rate of motion at the end of five hours. This was determined by obtaining the average time required for several individual trypanosomes to cross several microscopic fields. Since the diameter of the microscopic field was known, the route of each trypanosome observed is charted, measured, and the rate of motion is calculated in millimeters per minute. Five hours after drawing the blood was decided as the time of determining the rate of motion, because it was found that the influence of the standard solution began to show its effect about this time. Physiological saline was used as our standard solution. The trypanosomes appear to use up the small amount of fermentable carbohydrates and other essential elements contained in these 1 per cent solutions within five hours, and if there is no other available food, they will become slower in their motion and die very rapidly. That is what happens in the physiological saline solutions.

Table 4 shows the length of retention of virulence. This was determined by injecting equal numbers of motile trypanosomes into suscep-

TABLE 4.—*Table showing the effect on the virulence of* Trypanosoma equiperdum *after being held in vitro for various lengths of time.*

Animal number group	Days after inoculation of the trypanosome suspension									
	1	2	3	4	5	6	7	8	9	10
1. Group 1	—	+				D				
2. Group 1	—	+					D			
3. Group 2	—	—	+					D		
4. Group 2	—	—	—	+						D
5. Group 3	—	—	—	—	—	—	—	—	—	—
6. Group 3	—	—	—	—	—	+				D
7. Group 4	—	—	—	—	—	—	—	—	—	—
8. Group 4	—	—	—	—	—	—	—	—	—	—
9. Group 5	—	—	—	—	—	—	—	—	—	—
10. Group 5	—	—	—	—	—	—	—	—	—	—
11. Group 6	—	—	—	—	—	—	—	—	—	—
12. Group 6	—	—	—	—	—	—	—	—	—	—
13. Group	—	+			D					
14. Group	—	+		D						

Plus represents the day on which the first trypanosomes were demonstrated in the peripheral circulation (prepatent period).
D represents the date of death.

tible rats. The injections were made at five-hour intervals from the 1 per cent suspension of the solution and trypanosomes, namely: five, ten, fifteen, twenty, and so forth as long as living trypanosomes were seen. A fresh animal was used for each injection. The suspension in Locke's solution was considered the best. These results, in Table 4, are therefore from that suspension. At each subsequent injection the volume was increased according to the decrease in the number of motile trypano-

somes per cc. at time of injection. While most injections were made intraperitoneally, a few were made intracardially, but did not change the results.

In this experiment twelve rats were used. They were divided into six groups of two each. They were numbered one to twelve so that numbers one and two represented group one, while similarly eleven and twelve represented group six. The groups one to six were injected at five-hour intervals, so that group one received a five-hour-old suspension, while group six received a thirty-hour-old suspension. Animals numbers thirteen and fourteen were injected immediately after the organisms were removed from the host. They served as controls, since the time element was not a factor. In the table, plus represents the day on which the first trypanosomes were demonstrated in the peripheral circulation (prepatent period), while D represents the date of death. All rats in which the trypanosomes were demonstrated in the peripheral circulation eventually died from that infection. After fifteen hours of the trypanosomes *in vitro* no infection occurred upon their inoculation into susceptible rats, in spite of the increase in volume to compensate for the decrease in the numbers per unit volume.

Rats were more susceptible to insulin shock than guinea-pigs. Non-fatal shock was produced with one unit per 112 grams of body weight and fatal shock with one unit per eighty-nine grams of body weight. This unit for rats is near the standard unit for mice, which is one unit per one hundred eight grams of body weight (1/6 unit for an eighteen gram mouse). The fatal and non-fatal shock dose decreased correspondingly more rapidly in rats and mice during the course of the infection (4 to 6 days) than in guinea-pigs during their course of infection (10 to 17 weeks).

There was a difference in the changes in the differential cell count in the two sub-groups both in infected guinea-pigs and rats. This difference was chiefly in the endotheliocytes. The average increase during the acute phases of the infection in the non-insulin sub-group of guinea-pigs was from twelve per cent during the early stage of the infection, before the end of the prepatent period, to forty per cent at the stage where the trypanosomes were 39,100, which was about three weeks later. An increase to 26,000 over a similar period in the insulin sub-groups showed an endotheliocyte increase from twelve per cent before the end of the prepatent period to twenty-one per cent three weeks later. The changes in the rats were more rapid but less definite. Basophilia was observed in several of the younger infected rats, resulting in a five per cent basophilia. This high basophilia occurred only in young rats of six to ten weeks old. After the first two observations, a large number of differentials were done on normal young rats. They were found to have 1.5 per cent to

2.5 per cent basophils. This increased, however, in some cases to 6 per cent during the 4 to 7 days of the infection with *T. equiperdum*. The normal basophilia per cent in adult rats was 0.5. The younger rats lived longer than the adults when a proportionate number of *T. equiperdum* was injected into them.

DISCUSSION

The pathological physiology of trypanosomes is not yet fully understood. In our results the absorbable carbohydrates appear to be of definite value in the trypanosome metabolism and influence the course and duration of the infection in experimental animals. In human beings this influence does not exist, according to the observation of Wormall (5), who thinks that the resultant metabolites, and the oxygen deficiency may be of greater importance. The atmospheric oxygen does not appear of great value in our *in vitro* experiments, since there was no decrease in the survival time of the group under anaerobic incubation.

Since the non-fermentable reducing substance in the blood of rabbits did not vary greatly, according to Dotti (6), it appears that the variation in the amount of insulin necessary to produce shock is dependent on the amount necessary to deplete the fermentable blood sugar. The liver damage which results from the infection and which interferes with glycogen liberation is also a factor.

The basophilia is not explained. It is higher in younger animals, which resembles the higher percentage of nucleated red cells. The basophilia is probably the result of the stimulation of infection of an active hematopoetic apparatus of the young animals.

SUMMARY AND CONCLUSION

Fermentable carbohydrates seem to play a very important part in the rate of multiplication, survival time, and virulence of trypanosomes. The decrease in the blood sugar by insulin injection decreases the rate of multiplication of trypanosomes, as shown by examination of blood taken from peripheral circulation. Infected animals injected with insulin survived longer than similarly infected animals without insulin. There was a decrease in the dose of insulin required to produce a shock as the course of the infection progressed. This corresponded with the decrease in the amount of available fermentable carbohydrate in the blood and to the liver damage.

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FURTHER OBSERVATIONS ON PRENATAL HOOKWORM INFECTION OF DOGS[1]

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The occurrence of prenatal infection with parasitic worms suggests a situation of considerable economic and public health importance. As far back as 1921, the possibilities of prenatally acquired helminthiases had been strongly enough indicated to warrant the appearance of general discussions of this subject in medical journals of both the United States (Cort, 1921) and France (Neveu-Lemaire, 1921). Although our information is still too limited to evaluate properly either the real importance of this phenomenon or the frequency and extent of its occurrence under natural conditions, there can be no doubt that it has been demonstrated more or less readily under experimental conditions in a number of host-parasite relationships. It is significant also to note that many of the recent contributions to our knowledge of this phenomenon were anticipated in the early discussions mentioned above.

It has been shown experimentally that puppies can become infected before birth with the round-worm, *Toxocara canis* (Fulleborn, 1921; Shillinger and Cram, 1923; Augustine, 1927), and with the hookworm, *Ancylostoma caninum* (Adler and Clark, 1922; Foster, 1932). On the basis of animal experimentation, it appears that intra-uterine infestation with the Japanese blood fluke, *Schistosoma japonicum* (Fujinami and Nakamura, 1911; Narabayashi, 1914, 1916), has also been demonstrated.

In addition to these, there are many helminths for which observational evidence has been presented to indicate the probability of prenatally acquired infestation by both human and animal hosts. In this group are the human hookworm, *A. duodenale* (Howard, 1917; De-Langen, 1923), the agent of hydatid disease, *Echinococcus granulosus* (Deve, 1918), the cattle round-worm, *Ascaris vitulorum* (Macfie, 1922; Griffiths, 1922), the hookworm of the pig, *Necator suillus* (Ackert and Payne, 1923), the sheep lung-worm, *Dictyocaulus filaria* (Neveu-Lemaire, 1912), and the dog heart-worm, *Dirofilaria immitis* (Shipley, 1894; French, 1899; Hopkins, 1905). It is possible also that prenatal infection may occur with *Strongylus vulgaris,* since verminous aneurysms have several times been reported in foals which were too young to have developed such an advanced lesion after birth (Hutyra and Marek, 1926).

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Our present information suggests that the possibility of prenatal infection with any parasitic worm is conditioned upon (1) a blood-stream migration of the larvae, and (2) a tendency of the larvae to penetrate tissue barriers. It might be of interest to point out certain of the human helminths in addition to those discussed above which might be expected, on this basis, to be capable of infecting man prenatally. Among the nematodes, it appears that the above conditions are satisfied by *Ascaris lumbricoides, Necator americanus,* and *Strongyloides stercoralis.* It is not known that the larvae of *Trichinella spiralis* possess either the tendency or the ability to migrate from the blood stream, and it seems quite possible that they are transported to their final seat of encystment while still within the finer vessels. This may account for the apparently negative results of experiments which have tested for the possibility of intrauterine infection with this form. Among the cestodes, other than *Echinococcus,* it is possible that prenatal infection might rarely occur in the case of *Taenia solium.* This possibility is limited to the development of the cysticercus stage which presumably would become established in a fetus only if an onchosphere traversed the placental barrier. Other than the schistosomes it appears that the only trematode of man with which prenatal infection might occur, is the oriental lung fluke, *Paragonimus westermani.* Since the larvae of *Paragonimus* migrate through the tissues rather than by way of the blood stream and are larger than larval schistosomes it is improbable, although perhaps not impossible, that prenatal infection would occur with this form.

The present study includes additional data on the occurrence of prenatal hookworm infestation in dogs. In an earlier report (Foster, 1932) evidence was presented to show that all of the puppies of two litters (numbering 5 and 8 pups, respectively) born to mothers infected with *A. caninum* larvae during pregnancy acquired prenatal infestations. It was shown that in these cases the development of the larvae in the prenatally infected puppies did not take place until after birth. The fact that more than ten per cent of the larvae given the mothers during gestation were recovered from the puppies as mature worms was taken as indicating a wide-spread migration of hookworm larvae after oral infection, a phenomenon which it was suggested may have been associated with the resistance of the hosts. The marked susceptibility of the puppies born to very resistant mothers was pointed out to be difficult to reconcile with a serological explanation of this type of resistance. These findings made it desirable to pursue further the studies of prenatal hookworm infection, particularly since no difficulties were being experienced in the breeding and rearing of dogs and since both specific and nonspecific strains of the dog hookworm were available.

It was possible to obtain three litters of puppies from bitches which

were given only one dose of infective larvae during pregnancy and a fourth litter from a mother which was infected during late pregnancy with larvae of the "cat strain" of *A. caninum*.[2] The data from these experiments offer some interesting comparisons with the earlier results since in those cases reported previously the mothers were subjected to repeated infection during pregnancy.

The mother of the first litter had been given a total of 12,000 larvae *per os* over a period of 380 days, of which number, 5,000 were given in a single infection nine days before the puppies were born. She had been freed of worms from earlier infections, however, by anthelminthic treatment during pregnancy and the infective dose noted above was the only one given during this period. Two puppies were born and they were examined individually by the Lane Method on successive days, beginning seven days after birth. On the eleventh day one became positive to this examination, while the other did not. On the twelfth and thirteenth days the same condition prevailed and it was thought that only one of the puppies had acquired a prenatal infection. Since it was not necessary to autopsy these puppies in order to establish the fact that one of them was prenatally infected, they were kept available in case some additional experimental procedure should suggest itself. However, when these puppies were eighty days old, it was decided to autopsy the one which had become positive to Lane examination on the eleventh day. Only six hookworms were found. Finding this very light infestation made it desirable to obtain an autopsy record on the other pup of this litter, and in this case seven hookworms were recovered, one female and six males. It is not known whether this dog had been passing eggs immediately before autopsy but it seems reasonable to suppose that this infestation had been present since birth. Certain findings on the next litter indicate that the inability to recover eggs from this light infestation during the course of the early examinations might be explained by the possibility that the only female worm of the infestation was in copula during that time.

The second litter presented an interesting case, similar to that of litter 1, since in this instance also, a single infection given the mother produced very light infestations in the puppies. The mother of this litter was born in the laboratory March 30, 1931, and had successfully reared two litters previous to the one under discussion. This, her third litter, was born January 28, 1933. During the five months immediately pre-

[2] The so-called "cat-strain" of the dog hookworm, described by Scott (1929, 1929a, 1930) is a physiological variety of *Ancylostoma caninum*, the larvae of which are only slightly infective to dogs, but highly infective to cats. There is also a marked age resistance in the dog to infection with this strain, adult dogs being refractory to parasitism except under abnormal conditions, such, for example, as deficient diet (Foster and Cort, 1932; Foster and Daengsvang, 1932).

ceding this date, she had been given a total of 4,200 larvae in several doses but was freed of worms from these infections by anthelminthic treatment during pregnancy. It is apparent that at this age and under these conditions of infection she had become highly resistant to hookworms. A single infection of 2,000 larvae was given five days before the birth of 6 puppies. The mother developed a very light infestation showing an egg output of 25,000 to 50,000 eggs per day, which indicates a high degree of resistance although not a complete immunity. One of the puppies was killed and autopsied immediately after birth and a few hookworm larvae were recovered from the tissues with the use of the Baermann isolation apparatus. A second puppy was autopsied when nine days old and no worms were recovered, although an extended search was made. This does not necessarily mean that worms were not present, since at this age the developing worms were too large for recovery with the Baermann apparatus and too small to be seen easily with the unaided eye. Although a heavy infestation would not have escaped detection, it is fair to say that the finding of a 9-day-old larva, if only a few were present, by examination of the macerated organs, would have been largely a chance discovery. Of the other four pups, three became positive to Lane examination on the eleventh and twelfth days. The fourth puppy continued to be negative when examined by the Lane method on successive days and was sacrificed on the sixteenth day. At autopsy, four worms were recovered (1 female, 3 males) and the absence of eggs in the stools may possibly be accounted for by the fact that the only female worm of the infestation was in copula. A fourth puppy of this litter was autopsied at 19 days of age and three worms were recovered (2 females, 1 male). It was considered unnecessary to sacrifice the remaining two members of the litter since the presence of worms was already known from the Lane examinations.

The third case was one in which a mature female dog, which had never before been infected, was bred and then given a single infection of 1,500 larvae thirty-two days before the birth of eight puppies. The interest in this case centered upon whether or not an infection given so early in pregnancy would result in a prenatally acquired infestation of the puppies. One of the puppies was autopsied when six days old and one each of ascaris and hookworm larvae were recovered from its tissues. The other seven were examined individually by the Lane method on successive days. Five of these became positive on the eleventh day and another one on the twelfth day. The infestations in these six puppies were undoubtedly light as indicated both by the Lane examinations and by the fact that the puppies were enjoying good health. These puppies were not autopsied as it was felt that the additional information to be obtained thereby would not justify the sacrificing of so valuable an ex-

perimental group. The eighth puppy was autopsied, however, on the thirteenth day and no worms were recovered.

The fourth case is the record of an infestation among a single litter of puppies which has been regarded, in many respects, as the most convincing as well as the most interesting experiment of this series. The mother was given *per os* a single dose of 9,000 larvae of the "cat strain" of *A. caninum* two days before the birth of five puppies. Although this dog had previously harbored hookworms, she was treated to negative in early pregnancy, after which she remained negative to Lane examination, and no infections were given except the one listed above. In addition to the fact that this strain of *A. caninum* is only slightly infective to dogs it has been found also to have a much longer prepatent period than is encountered with larvae of the straight dog strain. In this instance, the prepatent period of the "cat strain" in the mother was nineteen days, as she began passing eggs seventeen days after the puppies were born, showing a very light infestation for a while thereafter. Meanwhile, all five puppies had become positive to Lane examination when from fourteen to sixteen days of age. The prepatent period in the puppies was, therefore, shorter than that in the mother and every puppy had shown evidence of infestation with mature worms before the mother had begun to pass eggs. One of these puppies died when 56 days old, while the rest were killed and autopsied at various intervals between 41 and 73 days of age. The worm burdens were found to be uniform throughout the litter, varying from 19 to 27 worms per dog, with an average of 24 hookworms each.

DISCUSSION

On the basis of the observations described above, it seems reasonably clear that prenatal infestations with the dog hookworm, *A. caninum*, were established in the puppies of four litters. Up to the present, the author has studied the condition of infestation of 34 pups (including data from previous paper, *loc. cit.*), representing six litters, and 32 of these puppies were found infected with hookworms. The circumstances giving rise to such infestations were those of repeated or single exposure of resistant bitches to infective larvae during pregnancy. In previous experiments (Foster, 1932, pp. 114–115) it has been shown that the presence of maternal infestation during pregnancy and nursing is not a factor giving rise to prenatal infection. It appears, however, that exposure of the mother to infective larvae as early as 32 days or as late as 2 days before parturition (gestation normally 63 days) may result in intra-uterine infestation of the puppies.

Considerable significance has been attached to the fact that the puppies born to resistant mothers showed a marked susceptibility to infec-

tion. In his study of prenatal infestation with the dog Ascaris Augustine (*loc. cit.*) stated that of the two bitches infected during pregnancy, one showed no worms at autopsy, and the other only a few worms. The puppies born to these bitches, however, harbored heavy infestations. He looked upon this as indicative of an age immunity in the bitch against this parasite, the larvae showing a decided preference for fetal tissues. The same condition of maternal resistance prevailed in the studies of Shillinger and Cram (1923) and of Fulleborn (1929). The latter wrote in connection with the findings in his own case, that the intestine of the mother dogs, in consequence of "immunity," was usually not infected (1929, p. 23). It is significant that, in all these studies, there was a lack of resistance, even prenatally, in puppies born to very resistant mothers. On the assumption that the resistance of these bitches was effected by serum antibodies one would have anticipated a temporary protection of the puppies. On the other hand, if it be assumed that the maternal resistance was effected primarily by age, one would have expected a condition of susceptibility among the puppies. This observation has been regarded as lending some support to the view that the resistance encountered in these host-parasite relationships may not be of a serological nature.

The results suggest also that there may be a relationship between exposure to infection with the human hookworms during pregnancy and infant morbidity and mortality.

ABSTRACT SUMMARY

The administration *per os* of single doses of infective larvae of *A. caninum* to three pregnant bitches at intervals of 5, 9, and 32 days, respectively, before parturition resulted in prenatal hookworm infestations in all but one of twenty-one puppies. Although the mothers were very resistant to hookworms, the puppies did not appear to be protected on this account. One litter of five pups was prenatally infected with the "cat strain" of *A. caninum* as a result of administering larvae to the mother only two days before birth of the puppies. It is suggested that the occurrence of prenatal infection with parasite worms may be of economic and public health importance.

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RESEARCH NOTES

DESCRIPTION, INCIDENCE, AND CULTIVATION OF *TETRATRICHOMASTIX BLATTIDARUM* N. SP. FROM THE COCKROACH

Description. The flagellate *Tetratrichomastix blattidarum* n. sp. has been observed in the intestine of three species of cockroaches, viz., *Blatella germanica, Blatta orientalis,* and *Periplaneta americana.* The flagellates were found in the posterior part of the intestine near the anus and occur in large numbers, when present. The greater the number occurring, the more anteriad they were found.

Figure 1. *Tetratrichomastix blattidarum* from the cockroach. ×2300.

T. blattidarum is pyriform-shaped, rounded at the anterior end and pointed at the posterior extremity (Fig. 1). A slender axostyle protrudes from the posterior end and extends from 1.5 to 3 μ beyond the body, with an average of about 2 μ. The body length, exclusive of the axostyle, ranges from 8 to 14 μ with an average of about 9 μ, the width varies from 4 to 8 μ with an average of about 5.5 μ. These measurements were made on 18 specimens which had been fixed with osmic vapor. There are five flagella, four of these are directed forward and the fifth is directed backwards. The anterior flagella are slightly longer than the body and the backward-directed flagellum is about twice body length. The flagella converge and enter the body slightly to one side of the anterior tip of the body. An indentation can be seen clearly in favorably stained specimens at the point of entry of the flagella. The flagella are difficult to count in living specimens but stain red in Giemsa and can be discerned readily. The nucleus lies in the anterior fourth of the body and often in close proximity to the anterior body wall. In haematoxylin stain, destained with picric acid, the nucleus shows a dark-staining endosome surrounded by a light-staining homogeneous area which fills the space between the endosome and the nuclear membrane. The axostyle, which stains poorly in haematoxylin but bright red in Giemsa, can be followed from the posterior end to the base of the nucleus and in one specimen seemed to divide and encircle the nucleus continuing on to the base of the flagella. The exact relationship of the flagella to the blepharoplast has not been determined. Blepharoplasts were seen in some specimens and in others the flagella seemed to be in direct contact with the nuclear membrane. The cytoplasm is vacuolated;

the vacuoles at times seem to occupy a large area of the body. Various types of bacteria were observed in the body, the predominating type being bacilli. The number of flagella is an important morphological feature, and since *Eutrichomastix* possesses three anterior flagella and the subgenus *Tetratrichomastix* four anterior flagella, it seems desirable to raise the latter to generic rank.

Incidence. Cockroaches of three species were examined to determine the incidence of infection. The *Blatella germanica* were secured in our laboratories, the *Periplaneta americana* in tunnels under the building, and the *Blatta orientalis* in the basement of a nearby residence. Of 51 *Blatella germanica* examined, 31 were parasitized, or 60.78 per cent. Twenty-two specimens of *Blatta orientalis* were examined and 16 of these harbored the parasite, an incidence of 72.72 per cent. Of 140 *Periplaneta americana* examined, 108 were parasitized, showing an incidence of infection of 77.14 per cent. The total number examined was 213, and out of these 155 were parasitized, a percentage of 72.77.

Cultivation. *Tetratrichomastix blattidarum* were inoculated into five types of culture media, viz., egg-mucoid, Doeck-Drohalov, Loefflers's serum-saline (Andrew's modification), Tanabe, and haemoglobin-saline. These were examined daily. The first three media showed no indications of sustaining growth. On the third day a very few flagellates were seen in the Tanabe but abundant growth never occurred. It is possible that the animals observed were some of the original inoculum and that successful multiplication and growth never occurred.

However, the haemoglobin-saline media showed substantial growth and seems to be a very satisfactory medium. They grew well in the original cultures and have grown through at least 18 subcultures. After inoculation, the animals multiply very fast being quite abundant on the third day and remaining in goodly numbers over a long period of time. They have been kept successfully by subculturing every two weeks. The favorable influence of this medium is shown by the fact that a culture has been maintained in a test tube for 27 days. The cultures were incubated at room temperature. Cultures did not survive at 37° C. The cultures were kept in test tubes plugged with cotton. In making subcultures, usually 0.5 cc. of inoculum was put into 4.5 cc. of culture medium.—MARTIN D. YOUNG, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

GIARDIA ENTERICA (GRASSI, 1881), THE CORRECT NAME FOR THE *GIARDIA* OF MAN

When Lambl in 1859 (Lambl, W., Vierteljahrschr. prakt. Heilk., 61: 1–58) described the *Giardia* of man he named it *Cercomonas intestinalis*. As pointed out by Kofoid and Christiansen, since the specific name *intestinalis* was preoccupied in the genus *Cercomonas* by Diesing's (1850) reference of *Bodo intestinalis* to that genus, Lambl's 1859 use of the name for the species from man is invalid. Grassi in 1879 (Grassi, G. B., Gazz. Med. Ital. Lomb., 39: 445–448) described the *Giardia* of the mouse and named it *Dimorphus muris*. In 1881 the same author (Grassi, G. B., Gazz. degli Osp., 2: 575–580) in his paper: "Di un nuovo parassita dell'uomo *Megastoma entericum*," gave the name *Megastoma entericum* to the organisms belonging to genus *Giardia* which he found in man, *Mus, Arvicola* and cat.

Kofoid and Christiansen in 1915 (Kofoid, C. A. & Christiansen, E. B., Calif. Pub. Zool. 16: 23–29) suggested the name *Giardia lamblia* Stiles in *litt.* for the species generally known as *Lamblia intestinalis* (Lambl) from man. In 1920, Kofoid (Kofoid, C. A., Univ. Calif. Pub. Zool., 20: 145–168) stated that "since the species in the mouse is distinct from that in man the name *Giardia muris* is applicable only to the former. No data are available as to the status of the form in *Arvicola* and infection in the cat is presumably derived from that in mice. By elimination the specific name *Giardia enterica* (Grassi) becomes available for the

Giardia from man in place of *intestinalis*. The name *Giardia lambla* Stiles (*in litt.*) proposed in Kofoid and Christiansen (1915) as a substitute for the preoccupied *intestinalis* becomes therefore a synonym of *Giardia enterica*."

Simon in 1921 (Simon, C. E., Amer. Journ. of Hyg., 1: 440–491) accepted the name *Giardia enterica*, "which according to the rules of nomenclature, would be correct, providing that the mammalian genus *Giardia* is, indeed, identical with that originally described in tadpoles, and to which Künstler in 1882 and 1883 first applied the name *Giardia*."

Boeck and Stiles in 1923 (Boeck, W. C. & C. W. Stiles, Hyg. Lab. Bull., 133, Wash. U. S. Pub. Health Service) observed that Grassi in 1881 changed to *entericum* the specific name *muris* previously (in 1879) given by himself to the parasite of the mouse. They based their statement on the following phrase of Grassi: "Perchè la denominazione *Dimorphus* potrebbe dar luogo a un equivoco e l'altra *muris* è ora diventata insufficiente, io credo opportuno di abbandonare quei nomi e di sostituirvi *Megastoma entericum* (mihi)." Boeck and Stiles emphasize that "it is seen that *Dimorphus* is renamed *Megastoma* and *muris* is renamed *entericum*; accordingly *muris* (s. *entericum*) becomes the type of *Megastoma*, and on the same principle the genus *Mus* (type host of *muris*) becomes the type host of *entericum*." So that in rejecting the specific name *enterica* as a synonym of *muris*, they conclude: "*Giardia lamblia* (according to Kofoid and Christiansen) was suggested by Stiles in a letter to Kofoid. Stiles has no record of this letter, but accepting the statement as correct, *lamblia* would stand as dating from 1915 when first published."

Now *Giardia lamblia* is used as correct by many authors, and in Wenyon's Protozoology, while the name *Giardia intestinalis* is retained as generally better known, *Giardia lamblia* is considered the correct name according to the rules of nomenclature. Nobody has until now pointed out that in Grassi's 1881 paper the specific name *Megastoma entericum* is first clearly referred to the parasite of man only. The title of the paper is: "Di unnuovo parassita dell' uomo *Megastoma entericum*," which means exactly: "Upon a new parasite of man *Megastoma entericum*." By the title man becomes immediately the type host of *Megastoma entericum*. In the contents of the same paper, Grassi applied the same name *Megastoma entericum* also to parasites of *Mus*, *Arvicola*, and cat believing them to belong to the same species. Since these parasites are now generally considered to be different species, the name *Giardia enterica* must be referred to the human parasite only, as indicated in the title of Grassi's paper. The specific name *entericum* given by Grassi to flagellates belonging to genus *Giardia* found in *Arvicola* and cat is not available for them as preoccupied by the organism of man, which is the only one reported in the title; while the specific name *entericum* given to the parasite of the mouse is unavailable since it is preoccupied by the parasite of man and is a synonym of *Giardia muris*. Therefore *Giardia lamblia* Stiles, 1915, becomes a synonym of *Giardia enterica* Grassi, 1881, and this latter name should be used as the only correct name according to the rules of nomenclature.—Augusto Corradetti, *Rome, Italy*.

MOLLIENISIA CAUCANA (STEINDACHNER) A LARVIVOROUS TOP-MINNOW FROM VENEZUELA

Specimens of a poeciliid fish collected in Pampan, Trujillo, Venezuela, were identified at the U. S. Bureau of Fisheries as *Mollienisia caucana* (Steindachner). Its ecology is similar to that of species of *Gambusia*. The fish are viviparous and live in shallow collections of water, especially among grass and in artificial reservoirs. They are so well adapted to these situations that it is not unusual to find them swarming with these minnows. In places frequented by ducks the fish are not found. In the pools where they abound, no mosquito larvae were present, though neighboring pools free from fish contained large numbers of larvae. A female of the species after having been without food for nearly 12 hours ate

100 fourth-stage culicine larvae in 5 minutes. These observations suggest that *Mollienisia caucana* might be utilized as an effective regional larvivore in anti-mosquito campaigns and in malaria control projects.—ARNOLDO GABALDON, *Department of Protozoology, Johns Hopkins University School of Hygiene and Public Helath.*

INFECTION OF ABNORMAL HOSTS WITH THE MOUSE STRAIN OF *HYMENOLEPIS FRATERNA*

In connection with studies on the dwarf tapeworm I had the opportunity to compare the percentage development of cysticercoids and adults of the mouse strain of *H. fraterna* in the white mouse, which appears to be the natural host (Shorb., 1933, Amer. Jour. Hyg., 18: 74–113), with the development in the rat, the deer mouse (*Peromycus maniculatus*), and the guinea-pig. These animals were 2½, 2, 1½ and 1 month of age, respectively at the time they were infected.

In each experiment about half of the animals were autopsied 93 hours after infection and the number of cysticercoids determined by a method described previously (Hunninen, 1935, Jour. Parasit., 21: 124–125) and the others were autopsied on the fifteenth day and the adults counted.

Of the 17 white mice, seven received 6,000 eggs and 10 received 5,000 eggs each. At autopsy 93 hours later in three of those receiving 6,000 and in four of those receiving 5,000 eggs, the percentage development into cysticercoids was 7.4. The other 10 mice were autopsied on the fifteenth day for adult worms and showed an average percentage development of 5.3. Although these percentages are slightly higher than the average found in a more extensive study, they fall well within the range of variation.

In the 16 black-hooded rats, which received 3,500 eggs each, the average percentage development into cysticercoids in eight of the animals was 3.8. In the other eight the average development into adults was 0.4. The adults were found in five of the eight rats and in all except one the worms that were recovered on the fifteenth day were very small and immature. Shorb (1933) found that an average of 0.49 to 2.5 per cent of the mouse strain eggs developed into adults in mice while in rats this average was only from 0 to 0.03 per cent. He also found a retardation in growth of worms in the rats.

In eight deer mice, each receiving 5,000 eggs, the average development into cysticercoids in four of these was 6.9. On the fifteenth day only one of the other four mice was positive with only two adults, giving a percentage development of 0.008. Both of these worms were small and immature.

Nineteen guinea-pigs were each infected with 5,000 eggs. Thirteen of these showed an average of 4.1 per cent dvelopment into cysticercoids. No adults were found in the other six animals. Guinea-pigs, however, are not totally refractory since Ransom (1921, Jour. Parasit., 7: 188) observed about a dozen immature worms of this species all less than 3 mm. in length from a guinea-pig which had been kept with rats.

From these experiments we can see the specificity of the adult worm and the lack of specificity of the cysticercoids. Owing to the wide range of insect intermediate hosts of the dwarf tapeworm in the indirect life cycles reported by Bacigalupo (1932, Revista Chilena de Historia Natural, 36: 144–147) in Argentina we should not be surprised to find so many mammals that can also serve as intermediate hosts to the strain of this worm that has adapted the definitive host as its intermediate host. From the results of these experiments we could predict that a still greater number of vertebrates will serve as intermediate host for the cysticercoids of the dwarf tapeworm.—A. V. HUNNINEN, *School of Hygiene and Public Health, Johns Hopkins University.*

EFFECTS OF A HIGH PROTEIN DIET ON *TRICHOMONAS HOMINIS* IN RATS

Previous experiments indicate that a diet rich in animal or plant proteins brings about a condition in the cecum of rats that is unfavorable for rat trichomonads. (For literature refer to Hegner and Eskridge, Amer. Jour. Hyg., 21: 135–150, 1935). During the past year we have been able to eradicate all of the intestinal trichomonads from laboratory rats with carbarsone and to infect them by means of oral inoculations with *Trichomonas hominis* from man. This has made it possible for us to study the effects of a high protein diet on *T. hominis* in rats.

Thirty male albino rats from 2 to 3 months old with an average weight of 258 grs. that were all infected with rat trichomonads were treated with carbarsone as in previous experiments. During one week after treatment pellets from each rat were examined daily by smear and culture and all were found to be negative. Ten of these were kept as trichomonad-free controls and were fed on a "normal" diet. They were killed 36 days later and all were found to be free from trichomonads by smear and by culture.

Ten of the rats that had been treated with carbarsone were each inoculated orally with 5 cc. of a culture containing 355,000 specimens of a pure line of *Trichomonas hominis* and fed on a "normal" diet. Pellets squeezed out of the rectum into culture medium in a test tube and incubated for 3 days at 37° C. all gave positive cultures. These rats were killed 36 days later; 9 of them were found to be positive by smear and culture. The total numbers of trichomonads in the cecal contents of these 9 rats were as follows: 4,800,000; 1,020,000; 1,020,000; 900,000; 150,000; 120,000; 60,000; 42,000; 42,000. The average number per rat was 906,000. The cecal contents were weighed and the numbers per gram determined as follows: 166,000; 92,727; 64,285; 46,363; 6,521; 6,000; 3,529; 2,625; 1,333. The average per gram of cecal contents was 43,265.

Ten of the rats that had been freed from their trichomonads with carbarsone were each inoculated with 5 cc. of the same culture as the group described above containing 355,000 trichmonads. Pellet-cultures proved that they all became infected. These rats were fed on a diet high in animal protein. When killed 36 days later, 3 were positive by smear and 5 by cultures; no counts could, of course, be made on the 2 rats that were not positive by smear. The other 3 contained total numbers of trichomonads as follows: 3,900,000; 480,000; 120,000. The average was 1,500,000. The number of trichomonads per gram of cecal contents was 5,571,428; 171,428; 53,330. The average was 1,932,062.

These data indicate (1) that the 30 rats were freed from their trichomonads by the carbarsone treatment, (2) that all of the 20 inoculated orally with *T. hominis* became infected, (3) that only 1 of the 10 infected rats that were fed on a normal diet lost its trichomonads during a period of 36 days following inoculation and (4) that 5 of the 10 infected rats that were fed on a high protein diet became trichomonad-free and that 2 of these contained such a small number that they were not found in smears. The conclusion reached is that in most cases a diet rich in animal protein produces an unfavorable condition in the cecum of rats for the growth and multiplication of *T. hominis* from man.—Robert Hegner and Lydia Eskbridge, *Department of Protozoology, School of Hygiene and Public Health, Johns Hopkins University.*

MINIMUM TIME REQUIRED BY *TRICHINELLA SPIRALIS* TO PRODUCE INFECTIVE LARVAE

The question as to the minimum age at which *Trichinella spiralis* larvae are infective was one that arose in connection with studies we have been conducting with this parasite. While it is difficult to ascertain the exact age of the larvae it was not necessary to do so in order to determine how soon after exposure to infestation one rat would become infective to another.

Seven laboratory reared rats were given 5000 larvae each by use of a stomach tube. Fifteen days later and on each succeeding day for six days one infected rat was killed, skinned, eviscerated and fed to two non-infected laboratory reared rats. Pressed diaphragm examinations were made of each of the infected rats before feeding. On the seventeenth day and thereafter, larva were microscopically visible in the diaphragm. This was coincident with the time of their establishing infection in the rats to which they were fed. One of the two rats to which the rat which had been infected for 17 days was fed died eleven days later and an examination of blood from the heart showed larvae.

The other thirteen rats survived and were autopsied thirty days after exposure. The four that had been fed rats previously infested for fifteen and sixteen days, respectively, failed to reveal any larva indicating that this period of time is not sufficient for the new generation of larva to become infective. Rats that had been infected for seventeen to twenty-one days contained larva that produced infections in laboratory rats.—L. O. Nolf and J. M. Edney, *University of Iowa.*

NIPPOSTRONGYLUS MURIS IN THE DEER MOUSE, *PEROMYSCUS MANICULATUS*

Nippostrongylus muris (Yokogawa, 1920) was originally described from the Norwegian rat *Epimys rattus norvegicus* taken from the Baltimore dumps. This nematode has also been found in the mouse *Mus musculus* caught by Mr. Conrad Bauer in the same locality and experimental infections of the white mouse have been reported by the author (1934, Proc. Helm. Soc. of Wash., 1: 13). In connection with the studies on this parasite in laboratory rats and mice the following tests were performed to determine if it would develop in deer mice (*Peromyscus maniculatus*).

Four deer mice 290 days old were each infected with 300 larvae and their feces examined daily by Lane D. C. F. All were negative for ova until autopsy on the twelfth day. No worms were recovered from the intestines but their lungs contained 1, 0, 4, and 11 larvae, respectively. Three white mice of the same age and infected with the same number of larvae harbored 100, 90, and 13 adult worms in the intestine and 13, 0, and 5 larvae in the lungs, respectively.

When it was observed that the larvae were able to reach the lungs, though not to grow to maturity in the intestine in these old deer mice, this test was repeated on younger animals. Two deer mice 40 days old were given 500 larvae each and at autopsy 13 days later no adults were found but 73 and 52 live larvae were recovered from their lungs. One deer mouse of the same age given 2800 infective larvae died four days later. Death was apparently caused by larval pneumonia as the lungs at autopsy were extensively hemorrhagic and congested (Porter, 1935, Jour. Parasit., 21: 226–228). Two hundred sixty-two larvae were recovered from the lungs of this animal and undoubtedly many more had passed on through. White mice infected with larvae from this culture were normally parasitized when autopsied.

From the results of these experiments it seems possible to conclude that *N. muris* does not reach maturity in the deer mouse. That it can migrate to the lungs and in cases of large numbers be pathogenic to young animals has been shown.—Dale A. Porter, *School of Hygiene and Public Health, Johns Hopkins University.*

A LARVAL *ECHINOCEPHALUS* IN A SEA URCHIN

Ward (1933, Jour. Parasit., 19: 262–268) discussed a parasitic nematode from a sea urchin with the statement that it would be described later. At his request I have prepared a brief note on this specimen, which was found by Dr. H. J. Van Cleave in the gonad of *Arbacia punctulata* at Woods Hole, Mass.,

May, 1917, and is now in the Ward Collection, No. 18.16. The worm is spirally coiled, forming about 2½ coils; its length is approximately 10 mm.; the width varies from 0.45 mm. near the anterior end to 0.7 mm. near the posterior end. Because of the immaturity of the specimen and discoloring of the cuticula little can be seen of the sexual features. In the structure and spination of the expanded anterior end, the absence of spines on the rest of the body, and the fine striation of the cuticula it agrees with *Echinocephalus uncinatus* as described by Baylis and Lane (1920, Proc. Zool. Soc. London, Sept., p. 245), and is tentatively assigned to this species. *Echinocephalus* larvae have not previously been reported from echinoderms; they have been found in bivalve molluscs, which are probably the usual intermediate hosts. The adults are parasites of skates and rays.—Sewell H. Hopkins.

THE NEMATODA AS PARASITES OF AMPHIBIA. II. CORRECTION

The writer (1935, Jour. Parasit., 21: 27–50) recently described as a new species a nematode from the intestine of *Triturus viridescens* collected near Woods Hole, Mass., under the name of *Capillaria brevicollis*. Through the kindness of Dr. M. C. Hall, Chief of the Zoological Division of the Bureau of Animal Industry, U. S. Department of Agriculture, my attention was called to the fact that the name *C. brevicollis* was a homonym of *C. brevicolle* Bittner, 1926, a parasite of the domestic goose. The name of *Capillaria brachyauchenia* n. comb. is therefore suggested for the parasite earlier described as *Capillaria brevicollis* Walton, 1935.—A. C. Walton, Knox College.

INFECTIVITY OF THE PLEROCERCOIDS OF *DIPHYLLOBOTHRIUM CORDICEPS* (LEIDY) FOR MAN[1]

Magath (1933, Jour. A. M. A., 101: 337–341), in discussing the public health relations of the broad tapeworm, *Diphyllobothrium latum*, referred to the fact that "Plerocercoids from trout of the Yellowstone have been fed to human beings without success." The experiment from which his statements was drawn was conducted by the writer at the U. S. Fisheries station in Yellowstone Park, Wyoming, in connection with investigations of the fish parasites of the native Yellowstone Lake trout (*Salmo lewisi*). Due to unfortunate publicity these trout have become more or less generally known as "wormy." This "wormy" condition is due to the infestation of a high percentage of the trout (70 to 80%) with the plerocercoids of *Diphyllobothrium cordiceps* (Leidy), the known adult hosts of which are the American white pelican (*Pelecanus erythrorhynchus*) and the California sea gull (*Larus californicus*). This "worminess" causes some concern among people fishing in this locality and many inquiries regarding this condition are made. One particularly persistent inquiry concerns the possibility of infestation of human beings by this worm.

The literature has little to say on the subject. Experimental workers in Oregon failed to infest dogs with *D. cordiceps*. Aside from this reference the writer has obtained no information.

In an attempt to throw light on the question, the writer, during July, 1931, ingested 8 viable plerocercoids, some free and some encysted. Examination of the feces for eggs were made at intervals over a period of more than two months. In December, 1931, 3 cc. of carbon tetrachloride was taken as an anthelmintic. No evidence was found pointing to development of the worm in the subject. The plerocercoids ingested were small, not above 7 mm. in length, and inasmuch as an experiment on the development in the normal host (the pelican) seemed to suggest that only the larger worms develop, a repetition of the experiment, using

[1] Published by permission of the Commissioner of Fisheries.

larger worms was carried out in July, 1932, 6 much larger plerocercoids, 20 to 70 mm. in length, being swallowed. Fecal examinations since then have failed to show any evidence of development of this worm. The evidence from these two trials would seem to indicate, although by no means conclusively, that *Diphyllobothrium cordiceps* (Leidy) is non-infective for human beings.

Since the above experiment was performed, Skinker (1932, Jour. Parasit., 19: 162), in a comparative study of *Diphyllobothrium cordiceps* and *D. latum*, concludes that "on the basis of morphology of the material available for study *D. cordiceps* for the present must be considered a synonym of *D. latum*." The above experiment suggests that while *D. cordiceps* may be morphologically identical with *D. latum* it may be physiologically a separate species. Simultaneously with Skinker's paper, Scott published a paper in which he concluded that the bothriocephalid in the Yellowstone bears is *D. cordatum*. If the plerocercoids in Yellowstone fish are those of *D. cordatum* they also should develop in man and dog. The negative evidence from Oregon and from the writer's experience does not support the idea that the Yellowstone bothriocephalid is either *D. latum* or *D. cordatum* but suggests that it may be a physiological species distinct from either one, although the evidence is insufficient to clearly establish the idea. Further studies are necessary to settle the question.—Lowell A. Woodbury, *Temporary Assistant, U. S. Bureau of Fisheries, University of Utah, Salt Lake City, Utah.*

INTESTINAL PARASITES OF ENGLISH SPARROWS IN ILLINOIS

In the course of a study of the parasites of the English sparrow, *Passer domesticus* L., 131 birds of this species were trapped in Champaign-Urbana and St. Joseph, Illinois, and examined by one of us (E. W.) with the results shown in the following table:

	1931			1932					
	Oct.	Nov.	Dec.	Jan.	Apr.	May	June	July	Total
Total No. Exam.	27	32	3	16	6	29	15	3	131
No. with Cestodes	3	3	0	0	2	1	0	0	9
No. with Coccidia	10	7	0	4	0	14	8	1	44

The incidence of infection was 6.9 per cent for cestodes and 33.6 per cent for coccidia. Of the 85 birds caught near the yards of the Animal Pathology Department, University of Illinois, where diseased poultry and other animals are confined, 38.8 per cent were infected with coccidia,while 24 per cent of the 46 birds caught elsewhere harbored coccidia. All of the coccidia were found in scrapings from the intestinal mucosa; they were identified as *Isospora* but the species was not determined.

All of the 9 cestodes found were identified (by S. H. H.) as *Choanotaenia passerina* (Fuhrmann, 1907). This species apparently has not been found previously in North America. Fuhrmann originally described *C. passerina* from *Passer domesticus* and *Fringilla ruficeps* in Europe and Egypt. Johnston (1909, Jour. Proc. Roy. Soc. N. S. Wales, 43: 405–411) gave a more complete description of specimens from *Passer domesticus* in Australia. These two accounts do not agree exactly. Our material agrees very closely with Johnston's description, but differs from Fuhrmann's in several features, notably the bilobed form of the ovary in our specimens. However, Fuhrmann (1932, Mem. Univ. Neuchatel, 8: 1–381) accepts Johnston's identification of the Australian specimens as *C. passerina*; if this is correct our worms must also belong to this species. Our largest complete worm was 55 mm. long, nearly all the ripe proglottids of most specimens being found free in the intestine of the host.

In the bilobed form of the ovary and in several other features our specimens and Johnston's material resemble *Paricterotaenia parina* (Dujardin); this species, which is also found in *Passer domesticus,* was formerly placed in *Choano-*

taenia, but was placed by Baer (1925, Rev. Suisse Zool., 31: 529–548) in *Icterotaenia* Railliet and Henry, 1909, and by Fuhrmann (1932) in *Paricterotaenia* Fuhrmann, 1932 (=*Icterotaenia*), because of the "sacciform" structure of the uterus. The uterus in our material is "sacciform" in ripe proglottids containing only immature eggs, but later, after the eggs and embryos are fully developed, becomes divided into small chambers most of which contain only one egg, so that the eggs appear to lie in the parenchyma, as in other species of *Choanotaenia.* The form of the uterus seems to be a poor basis for the separation of genera in this group. It is not impossible that *C. passerina* and *P. parina* are actually synonymous, but we lack the type specimens necessary for determination of this point. Linton (1927, Proc. U. S. Nat. Mus., v. 70, Art. 7) has reported *P. parina* from the United States.

Choanotaenia passerellae Cooper, 1921 (Report of the Canadian Arctic Exped., 1913–1918, v. 9, Part G–H), which Baer places in *Icterotaenia* and Fuhrmann in *Paricterotaenia,* also shows a rather close resemblance to our specimens. Examination of Cooper's sections, now in the Ward Collection, shows that the uterus is divided into small pockets as in the rest of the genus *Choanotaenia*; therefore if the form of the uterus is a valid generic characteristic *C. passerellae* must remain in *Choanotaenia.*—Sewell H. Hopkins and Evan Wheaton.

TRANSMISSION OF *GIARDIA LAMBLIA* AND *G. MURIS* TO THE WOOD RAT

The possibility that rodents may act as carriers of the human intestinal Protozoon, *Giardia lamblia,* is important from a sanitary point of view. To explore this possibility tests have been made with the wood rat which not infrequently invades human habitations, especially in the vicinity of suburban and country residences. Experiments were carried out on a series of six wood rats—four from San Diego, *Neotoma fuscipes macrotis,* and two from Berkeley, *Neotoma fuscipes annectens.* The wood rat was chosen for these experimental infections in preference to cats, mice, and rats used by other workers since this rodent has not been found to carry a natural infection by any species of *Giardia.* Lavier (1924, Ann. Parasitol., 2: 161–168) failed in his attempts to infect laboratory rats with *Giardia lamblia* from man but found in sewer rats a *Giardia* morphologically similar to *G. lamblia* which he named *G. simoni.*

Previous to our transmission experiments a series of 30 normal wood rats from both localities was examined and determination of the species of Protozoa present and their distribution in the intestine made. In none of these rats was any species of *Giardia* ever found. Fecal examinations were likewise made of the six experimental wood rats but all proved negative for *Giardia.*

Four of these wood rats, having been proven negative for this flagellate, were then immobilized in a wire mesh cylinder and fed a saline emulsion of a three-day old human stool which contained cysts of *G. lamblia.* Two other wood rats received a saline suspension of caecal contents from the white rat, containing trophozoites and cysts of *G. muris.* Each rat received 1.00 cc. of the inoculum *per os.*

The rats were autopsied from one to three weeks following the feeding and in the four receiving the cysts from man there was found a very heavy infection of *Giardia lamblia,* and the two receiving the cysts from the rat had heavy infections of *G. muris.* The *Giardia* were found throughout the entire small intestine, from within one-half inch below the stomach to within three inches above the ileocaecal valve in both instances. However, the infection was definitely heaviest in the upper limits of the jejunum. This is in accordance with the results obtained by Kofoid, McNeil, and Bonestell (1933, Univ. Calif. Publ. Zool., 39: 179–190) on the distribution of *Giardia* in the Norway rat.

Hegner's (1927, Amer. J. Hyg., 7: 433–447) infections in laboratory rats with

G. lamblia decreased on the twelfth and fourteenth days and disappeared entirely on the sixteenth and nineteenth days. In the experiments here reported there was no diminution in numbers after the sixteenth day; on the contrary, the flagellates were abundant on that day. When the rats were killed on the twenty-first day after inoculation, *Giardia* was still present in very large numbers, great clumps filling the entire microscope field. There was no indication that the infection was dying out.

When infection with *Giardia* is very heavy the intestinal range of the parasites is extended so that they are to be found from the upper duodenum to the lower ileum. If the infection is light the duodenum and lower ileum are usually negative. In both cases the main focus of the infection is in the jejunum. In such heavy infections the surface of the duodenum and the upper jejunum is found to be thickly covered with the parasites. They lie on the tissue and in the interstices, motionless except for a slight beating of the flagella, and hence may be easily overlooked by the casual observer. At a point where the infection is heaviest, namely, the jejunum, great numbers may be seen swimming freely and rapidly about in the fluid of the dissected intestine, with but few actually attached to the tissue wall. Throughout the ileum the number of flagellates declines greatly and their activity decreases, many of them being practically motionless in the tissue fluid and none remain attached to the tissue wall.

The wood rats were kept on the regular routine rat diet as quoted by Kofoid, McNeil, and Bonestell (*l. c.*). Glukhowtzev (1935, Trans. Past. Inst. Epidem, and Bact. in Leningrad, 2: 52–81) found that a group of white rats fed on a pure meat diet and a group on a pure diet of fresh beer yeast all failed to become infected with *G. lamblia* and *G. muris*. However, he found that pure diets of milk, vegetables, fat, lactose, and mixed diets all created favorable conditions for infection with *Giardia*.

The pH of the intestinal tract of the wood rat is normally more alkaline than that of the Norway rat, as determined by means of the glass electrode by Kofoid, McNeil, and Bonestell (1935, Univ. Calif. Publ. Zool., 41: 1–8). Upon infecting wood rats with the human species of *Giardia* the pH drops down to a pH of 6.7–6.8 from 6.91–7.22. Normal cysts of both species appeared in the faeces of the experimentally infected wood rats.

These results suggest a possibility that rodents may be a factor, to an extent at present undetermined, in the spread of human giardiasis, especially in communities without sanitary disposal of sewage, by access to infected human stools and contamination of food supplies in homes, stores, and warehouses.—Aileen E. Bonestell, *University of California.*

NOTES ON THE LOUSE PROBLEM OF A MUNICIPAL WORKHOUSE

At the Minneapolis city workhouse from February 18, 1932, to October 31, 1933, a daily record was kept of the men who arrived infested with lice. During that period 7,818 men were examined of whom 23.8 per cent were infested. In a study of conditions in common lodging houses in London, Nuttall (Parasitology, vol. 10, p. 80–185, 1917) found "that there is an increase in the louse population in the winter months and a decrease in summer." The percentage infestation of the new inmates at the Minneapolis workhouse during 1932 agreed with this finding. In 1933 it appeared to be higher in the summer, especially in August when it went to 36.3 per cent, but data are not available to show the tendency in the latter part of that year. Nuttall reported that, in his examinations of beds in the London lodging houses, a maximum of 31 per cent were infested with lice in the early part of February. The minimum infestation (12 per cent) occurred early in June.

It was thought possible the percentage of infested men in the class we investigated could be correlated with the number of rainy days, either during the month under consideration or during the previous one. It is likely that indi-

vidual persons come in closer contact with each other and therefore are more likely to become infested with lice on such days, but this could not be shown with the available data.

In general the percentage of infestation was higher in 1933 than in the previous year. Perhaps economic conditions had something to do with this increase.

A certain man, aged 55 years, encountered in February, 1932, at the workhouse, admitted he had been a frequent visitor there, having run afoul of the law about fifty times prior to that month. Inspection of his trousers resulted in the collection of 454 mature lice within a half hour. In the crotch of his union suit as high as 117 nits per square inch were counted. Many nits and active lice were present on his shirt, coat and overcoat, especially under the arms. This infestation had been acquired in about 14 weeks. The man was discharged on April 10th, but was returned to the workhouse on May 9th, at which time several mature lice and many nits were collected from his clothes. On June 4th he was discharged the second time that year, but was brought back again on July 30th with a somewhat greater infestation gained during the slightly longer absence.

At the Minneapolis workhouse the clothing of the new arrivals is de-loused by heating it in an electric oven. The maximum temperature used is 248° F., about an hour being required to reach this point after the oven is turned on. After the temperature has been held automatically at the maximum for an additional one and one-half hours, the current is turned off and the oven, still containing the clothing, left closed until the next morning. It was found by wrapping a thermometer tightly in a bundle, that the temperature there had risen only to 106° shortly after the temperature in the oven reached 248°. Lice within a bundle, therefore, could survive if they were removed from the oven at that point. Two thermometers, each in a bundle of clothes, registered 148° and 158°, respectively, at the end of two hours, *i.e.,* after the temperature in the oven had reached 248° and remained there for about an hour.

It is very important for an oven operator to realize the time required for heat to penetrate heavy and bundled clothing. The contents of the oven should be packed as loosely as possible, so the heat will penetrate to all parts of it and kill lice wherever they may be hidden.—M. J. Oosthuizen and H. H. Shepard, *University of Minnesota, St. Paul, Minnesota.*

ACTIVE IMMUNITY IN RABBITS TO THE LIVER FLUKE, *FASCIOLA HEPATICA*

Serological studies on liver fluke infections led us to believe that it might be possible to induce an active immunity in rabbits against this parasite. After considerable difficulty we were able to establish the life cycle in the laboratory and secure enough metacercaria to infect our animals. We are indebted to Dr. J. N. Shaw for our supply of *Galba bulimoides* Lea, the intermediate host which we used.

Through the kindness of Dr. M. C. Hall and Dr. B. Schwartz we were able to secure a supply of living liver flukes isolated from the livers of cattle and sheep. The anterior ends of these were cut off, and the eggs were teased free from the fluke tissue and allowed to develop. The remaining part of the worms were washed thoroughly in saline solution and dried in an incubator at 55° C. After drying they were ground to a fine powder and stored in a desiccator.

Seven rabbits were subjected to a series of intraperitoneal injections of 1 cc. of a 1 per cent suspension of the dried powdered fluke material in physiological saline solution. The injections were made at intervals of one or two days over a period of three weeks, a total of nine injections being given. One, two and three weeks after the last injection positive precipitin tests were secured. These seven animals and three control rabbits were each infected with 13 metacercaria about 36 days after the last injection into the experimental rabbits.

Four of the experimental rabbits were autopsied 92 days after infection, one fluke was found in one of these (R 8), two flukes each in two rabbits (R 4 and R 6) and three flukes in the fourth rabbit (R 7). Two more rabbits (R C B and R 2) were autopsied 103 days after infection and two flukes were found in each. The seventh rabbit (R 9) was autopsied 118 days after infection and contained three flukes. An additional point of interest was noted in two cases, namely, the calcification of the parasites. In rabbit R 4 one of the two flukes present was calcified and in rabbit R 6 both of the flukes were calcified. No eggs were found in the feces of these animals during the course of the experiment although they were examined at weekly intervals from the 10th week after infection until their death, which occurred after eggs had appeared in two of the control rabbits.

The three control rabbits, no's. 2, 3, and 255, were autopsied at 124, 111, and 116 days after infection and 11, 5, and 9 flukes, respectively, were found in them. Thus, from two to ten more flukes were found in these rabbits than in the experimental animals. A second difference between the two groups was that none of the parasites found in the control rabbits were calcified. The third difference was that eggs were found in the feces of all of the control rabbits, in no. 2 from the 89th day, in no. 3 from the 76th day, and in no. 255 from the 108th day after infection until the death of the animals.

These differences between the two groups seem to indicate that it is possible to establish an immunity in the rabbits subjected to the injections of the dried fluke material.—K. B. Kerr and O. L. Petkovich, *Department of Zoology, Washington University.*

REVIEWS

ONCHOCERCA, with special reference to the Central American form of the disease. By Richard P. Strong, Jack H. Sandground, Joseph C. Bequaert and Miguel Munoz Ochoa. Harvard University Press, xiv, 234 pp., 1934.

This report embodies particularly the results of field and laboratory studies of the expeditions organized and conducted in 1931 and 1932 by the senior author for the study of the disease in Guatemala. The volume is divided into four parts written respectively by the above men in the order named. In Part I, Strong brings together a comprehensive review of the literature on *Onchocerca* and also draws freely upon his African experience in discussing the various medical, biological, and epidemiological aspects of the American, particularly the Guatemalan, form of the disease. He notes the marked localization of the disease on the Pacific slope of Guatemala and Chiapas Province, Mexico, with some extension further up the Pacific slope of Mexico. In Guatemala 95 per cent of the cases occur between 2500 and 5000 feet elevation and 98 per cent of the infected individuals are Indians. Despite the fact that the first reports of the disease in the Americas was in 1915, the author discredits the view that it may have been introduced from Africa by the negroes. Apparently the disease has never become established among the negroes in the Americas and occurs among the Indians where negroes are almost unknown.

In Part II, Sandground supports the senior author in this belief despite his evidence for the morphological identity of the African and American forms of the parasite. For the present apparently the origin of the human infestation in America must remain a mystery but Sandground has as he says "the temerity to suggest that an investigation of a possible bovine source is desirable." The authors agree that the biting habits of the flies and differential effect of protecting clothing and vegetation may account respectively for the cephalic and corporal distribution of the nodules in America and Africa. They note, however, that there is no proof that the worms mature and excite nodule formation at the site of the fly bite.

Strong also discusses the nodule formation and comments particularly on the histopathological aspects of the ocular complications. He further reviews what is known of the immunological and public health aspects of the infection and considers the possibilities of treatment and prophylaxis.

Sandground in Part II is particularly concerned with the taxonomic phases of the problem. As a result of careful studies of a variety of forms, he finds that there is no morphological basis for the specific rank of the two human forms and further that they are morphologically identical with most of the bovine and equine species described. He would, however, hold in abeyance the suppression of the several species "pending the outcome of comprehensive research on the subject." He summarizes the description given for the several species and notes that the only distinguishing feature so far given is the host and location in the host. He also gives a more adequate description of the genus than hitherto given.

Bequaert in Part III, considering the intermediate hosts, reviews the taxonomy, morphology, and biology of the Simuliidae. He suggests several theoretically possible methods of control for trial, particularly suggesting the use of heavy oils, such as Phinotas oil, in streams where the larvae and pupae develop. He views also the various repellents in local use for the protection of man and animals.

Ochoa in Part IV gives briefly certain specific data on epidemiological importance in Guatemala.

An excellent bibliography after each of the first three parts adds materially to the utility of this valuable book.—G. F. OTTO.

ARCHIVOS INTERNACIONALES DE LA HIDATIDOSIS. Vol. I, Fasc. I, Diciembre 1934. Montevideo.

The first number of this new journal on echinococcosis is published from the Centro de estudio y profilaxis de la hidatidosis, Ministry of Public Health of the Republic of Uruguay and edited by Professor Velarde Perez Fontana, Professor of Pathological Surgery of the University of Montevideo. As indicated by the title it is truly international in its content. The purposes of this journal are set forth in a foreword printed in Spanish, French, German, English, Italian, Portuguese, and Russian, quotations from which follow:

"The purpose of the Archivos Internacionales de la Hidatidosis, is to form a compendium of works and publications from all parts of the world dealing with Hidatidosis. Despite the progress achieved in the realm of Hydatic pathology, the corresponding prophylaxis has not yet been established on a systematic and definite basis.

"The Archivos Internacionales de la Hidatidosis, will be published periodically, and the scientific output and bibliography of every country will be collated annually in its own language, and dealt with in a Spanish summary. They will be supplied in exchange for other publications dealing with Hydatic diseases."

The first article deals with the circumstances leading up to the establishment of the department for the study and control of echinococcosis of the ministry of public health of Uruguay (Centro de Estudio y Profilaxis de la Hidatidosis del Ministerio de Salud Publica, Republica Oriental del Uruguay).

This is followed by an article on Felix Dévé, the great French authority on hydatid diseases, which includes a photograph and a bibliography of his contributions to the subject numbering 267 titles and extending from 1901 to 1934. Further biographical articles including photographs and bibliography are given for the following men who have made important contributions to our knowledge of echinococcosis: Alejandro Posadas, Daniel J. Cranwell, Marcelino Herrera Vegas, Ricardo Lozano, Harold R. Dew, Pedro Escudero, and Mariano R. Castex.

The number also includes reviews and original articles on hydatid disease and mortality statistics on this disease from the Republic of Uruguay.

Correspondence in regard to this publication should be addressed to Prof. Velarde Perez Fontana, Casilla de Correo No. 255, Montevideo, R. O. del Uruguay.—W. W. CORT.